Thermostable Bacterial Protease - A New Way for Quality Silk Production

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Abstract

Silk degumming process is a fundamental finishing process for silk yarn and silk fabric. The objective of degumming is remove the substrate such as silk gum (sericin), wax and some impurities from silk fibres. The principle of degumming process is breaking the peptide linkage of amino acid in sericin structure into a small molecule, which is soluble in water. The methods used for the degumming are the hydrolysis reaction performed by acid and alkaline, but these methods are not eco-friendly and same time they have a big problem on the surface area of silk. Hence the proteolytic enzyme have been used to solve this problem and shown promising results not only in the production level but also quality of silk. Since protease based degumming is eco-friendly which will be an additional advantage. Though the conventional protease are quite efficient for degumming but having some disadvantage like thermal and chemical stability which is one drawback has to solved, also alkaline protease can hamper the quality and physical appearance of silk as silk is quite sensitive to alkali and alkaline protease. The thermostable protease basically forms Geobacillus genus has been used for the enzymatic degumming of silk which are quite resistant to various chemicals and temperature. In this article we have summarized comparative analysis of detergent, conventional protease and thermostable protease for efficient degumming.

Keywords: *Silk production, Silk Degumming, Industrial Enzymes, Thermostable Protease, Enzyme production*

1. Introduction

1.1 Silk

The wild *Bombyx mori* silks are the strongest natural protein fibers and excellent fibers with many outstanding attractions, including luster, dyeability, moisture absorption. However, the silks still have disadvantages such as weakness when they are exposed to sunlight and changed to yellow, degradation when they are heated at high temperature, wrinkle when they are wetted, and these properties need to be improved. In recent years, graft copolymerization with vinyl monomer has become a well-known chemical finishing for improving new properties or heightening existing properties in the parent polymer.[1] The grafting techniques

for silk fibroin with different types of vinyl monomers, that is, methyl methacrylate (MMA) [2], methacrylamide (MAA) [3] and 2- hydroxyethyl methacrylate (HEMA) [4] have been reported. Moisture regain and acid-base resistance properties of HEMA grafted silk were higher than that of the degummed silk. Silk grafted by HEMA and MMA show improvements in thermal stability and dye uptake. [5]

In the present scenario, new process and products are linked with biotechnology directly or indirectly in through the various research and industrial fields. These are the most diverse possibilities for its profitable use in the textile industry. Enzyme is a biotechnology product, which are the new addition in the field of textile finishing. Many areas of textile enzyme finishing like cotton, linen, jute, and ray on have been conquered since then. [6,7]Today, prospects lie in the field of developing new durable enzyme finishes for silk. The conventional and thermostable protease enzyme is the best one followed by papain and trypsin. So these natural enzymes could be used for silk degumming effectively in industries as they have the added advantage of being eco-friendly in nature. [8]

1.2 Properties

1.2.1 Physical Properties

The easiest sources of silk fibres from the *Bombyx mori* silkworm have a triangular cross section with rounded corners, 5-10 μm wide. The fibroin-heavy chain is composed mostly of beta-sheets, due to a 59-mer amino acid repeat sequence with some variations. [9] The flat surfaces of the fibrils reflect light at many angles, giving silk a natural shine. The cross-section from other silkworms can vary in shape and diameter: crescent-like for *Anaphe* and elongated wedge for *tussah*. Silkworm fibers are naturally extruded from two silkworm glands as a pair of primary filaments (brin), which are stuck together, with sericin proteins that act like glue to form a bave. Bave diameters for tussah silk can reach 65 μm. See cited reference for cross-sectional SEM photographs.[10]

In the physical appearance silk has a smooth, soft texture that is not slippery, unlike many synthetic fibers. Silk is one of the strongest natural fibers but loses up to 20% of its strength when wet. It has a good moisture regain of 11%. [11] The mechanical parameter like elasticity is moderate to poor: if elongated even a small amount, it remains stretched. It can be weakened if exposed to too much sunlight. It may also be attacked by insects, especially if left dirty. Conduction of electricity is poor in silk fibre and thus susceptible to static cling.

The processed unwashed silk chiffon may shrink up to 8% due to a relaxation of the fibres macrostructure. Hence to avoid the following complication silk should either be washed prior to garment construction, or dry cleaned. During the dry cleaning silk fibers may still shrink the chiffon up to 4%. [12] Though, shrinkage can be reversed by a gentle steaming with a press cloth. There is not any evidence showing shrinkage at the molecular-level deformation. Both natural and synthetic silk are known to exhibit piezoelectric properties in proteins, probably due to its molecular structure. [13]

1.2.2 Chemical Properties

Silk emitted by the silkworm consists of two main proteins, sericin and fibroin, fibroin being the structural centre of the silk, and sericin being the sticky material surrounding it. Fibroin is made up of the amino acid Gly-Ser-Gly–Ala and form beta plated sheets Hydrogen Bonds form between chains, and side chains form above and below the plane of the hydrogen bond network. [14]

The high proportion (50%) of glycine, which is a small amino acid, allows tight packing and the fibers are strong and resistant to breaking. The tensile strength is due to the many interceded hydrogen bonds, and when stretched the force is applied to these numerous bonds and they do not break. Silk is resistant to most mineral acids, except for sulphuric acid, which dissolves it. It is yellowed by perspiration. [15]

Composition of silk in *Bombyx mori* [16]

Table 1: Composition of Natural Silk obtain form the Silk worm Bombyx mori

S.No.	Components	% Availability
1.	Fibroin	70-80
2.	Sericin	20-30
3.	Wax	0.4-0.8
4.	Carbohydrate	1.2-1.6
5.	Inorganic Matter	0.7
6.	Pigments	0.2

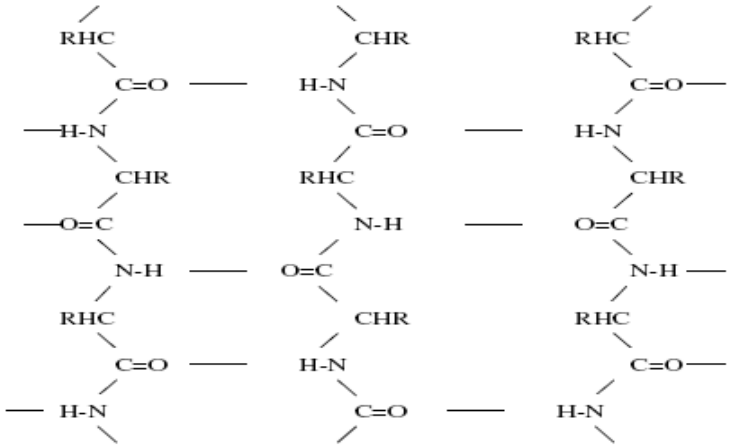

Fig:1 The following figure shows basic alignment of aminoacids in the silk protein Fibroin form silkworm *Bombyx mori* [17, 18]

1.3 Silk Production and Processing

For the commertial production of silk silkworms are cultivated and fed with mulberry leaves. The eggs are hatched by artificial means such as an incubator, and in the olden times, the people carried it close to their bodies so that it would remain warm.Silkworms that feed on smaller, domestic tree leaves produce the finer silk, while the coarser silk is produced by silkworms that have fed on oak leaves. From the time they hatch to the time they start to spin cocoons, they are very carefully tended to. Noise is believed to affect the process, thus the cultivators try not to startle the silkworms. [19]

Onces the coccon get ready then cultivators gather the cocoons and the chrysales are killed by heating and drying the cocoons.In the olden days, they were packed with leaves and salt in a jar, and then buried in the ground, or else other insects might bite holes in it. Modern machines and modern methods can be used to produce silk but the old-fashioned hand-reels and looms can also produce equally beautiful silk. [20] In the process of silk many steps runs in cascade manner in order to have a compelte process of silk production. Many process like removal of raw silk, washing, removal of impurities and dying and drying are bit essential but removal of wax and other unwanted protein form raw silk is most impartent step which make difference in the normal and quality silk known as degumming.

1.4 Silk Degumming

The silk filament secrete by the silkworm (*Bombyx mori*) is composed of two fibroin filaments held together by a cementing layer of sericin. Natural silk consist of protein fibers with two main parts called fibroin (fibre part 62.5 – 67.0 %) and silk adhesive called as silk gum, sericin (23.0 – 27.5 %). [21] Sericin acts as an adhesive for the twin fibroin filaments and conceals the unique luster of fibroin. Sericin contains some impurities such as waxes, fats and pigments. Thus, need to remove sericin (degum) that cover on the fibre surface in order to obtain a luster, soft handle and the other desired properties for further process. [22] The conventional method for the degumming of silk under alkaline conditions at a pH of 10 to 11 near boil has a long history. The pprinciple behind silk degumming process is increasing the silk gum solubility by breaking the peptide linkage of sericin structure into small molecule such as amino acid and its oligomer with hydrolysis reaction. [23] Silk degumming can be performed by numerous methods such as using alkaline and synthetic detergent. However, alkaline conditions are harmful to silk fiber because silk has poor resistance to alkaline. In the present scenario the proteolytic enzyme be used to solve this problem but it has some disadvantages about specific condition and high costs [24].

In recent years two new processes have emerged. They are the "H.T.-H.P. Degumming" and "Enzyme Degumming". [25] The new Technology; High temperature High Pressure degumming requires special pressured equipment and is energy intensive process. Enzymatic degumming is emerging as an eco-friendly fiber-gentle process where proteolytic enzymes that are effective under alkaline, neutral as well as acidic conditions are being used. [26] With the local availability of the enzymes at a reasonable price this process has a commercial potential in India. Being large molecules, enzymes do not penetrate into the interstices of the fabric and hence are suitable for yarn degumming only. A critical control of the pH and temperature is required to realize the full potential of the enzymes requiring use of sophisticated machinery such as the Mezzera yarn-dyeing machine. Since most of the enzymes are effective at a comparatively low temperature of about 600C, they are less energy -intensive. [27] The degumming waste liquor that is rich in sericin content is being used as a raw material for the production of sericin powder. The sericin powders are being used in the cosmetic industry as moisturizer, in hair-care products and also as a sustainable natural textile finish. Removal of sericin from the waste degumming liquor also substantially reduces the effluent [28].

1.5 Global Silk Production

The worldwide silk production in last one decade calculated more than 80000 tonnes and one third of which is produced in China India and Japan. The silk has been produced form

different source but around 20% form mulberry silk and China is main exporter of mulberry silk. The last decade the cost of silk was $ 40-50 per kilogram [29].

Table 2: The global silk production in last 10 year

S No	Country	Silk production in tons in last 10 year in tonnes
1.	China	290,003
2.	India	77,000
3.	Japan	5527
4.	CIS	4000
5.	Brazil	1903
6.	Others	3960

Table 3: Export of process silk in last 10 year

S No	Country	Silk Export in last 10 year in tonnes
1.	China	7919
2.	Brazil	1413

Table 3: Import of processed silk in last 10 year

S No	Country	Silk Import last 10 year in tonnes
1.	Japan	2756
2.	South Korea	2300
3.	Thailand	650
4.	Switzerland	149
5.	France	271
6.	Italy	2432
7.	United Kingdom	53
8.	United State of America	162
9.	India	2000

2. Historical Background

2.1 History of Silk

The Chinese has used silk since the 27th century B.C. During the Roman Empire, silk was sold for its weight in gold. The Chinese domesticated silk worms and fed them with mulberry leaves. They unwound the silkworms' cocoons to produce long strands of silk fiber. Silk is a way of life in India. Over thousands of years, it has become an inseparable part of Indian culture and tradition. No ritual is complete without silk being used as a wear in some form or the other. Silk is the undisputed queen of textiles over the centuries. [30] Silk provides much needed work in several developing and labor rich countries. It is one of the most labor intensive sectors of the Indian economy combining both agriculture and industry, which provides for means of livelihood to a large section of the population i.e. mulberry cultivator,

co-operative rearer, silkworm seed producer, farmer-cumrearer, reeler, twister, weaver, hand spinners of silk waste, traders etc. It is the only one cash crop in agriculture sector that gives returns within 30 days. This industry provides employment nearly to three five million people in our country. Sericulture is cultivated in Karnataka, Bengal, Tamil Nadu, Andhra Pradesh, Jammu & Kashmir, Gujarat, Kerala, Maharashtra, Uttar Pradesh, Rajasthan, Bihar, Orissa etc. [31]

Though India is the second largest silk producer in the World after China, it accounts for just 5% of the global silk market, since the bulk of Indian silk thread and silk cloth are consumed domestically. Germany is the largest consumer of Indian silk. The sericulture industry is land based as silk worm rearing involves over 700,000 farm families and is concentrated in the three Southern states of Karnataka, Tamil Nadu and Andhra Pradesh. (The states of Assam and West Bengal are also involved in the industry to a certain extent). Silk, the crown of the fabrics still commands passion of consumer right from 2200 BC to till today, nationally and internationally. [32] The export potential of Indian Sericulture Industry is evident from the fact that the annual export is Rs.2879.56 crores during the year 2004-05. The Central Silk Board, Ministry of Textiles, Govt. of India has been acting as a facilitator for planning, development and monitoring of sericulture industry between the States and Central Governments. [33]

3. Detergents in Silk Production

3.1 About Various Detergents

Soap Degumming

Soap has been used in the process of silk degumming with synthetic detergents implies the partial or total replacement of soap with synthetic non-ionic surfactants (e.g. ethoxylate fatty alcohol). To achieve the better yield and quality soaps have been combine with an oxidizing or reducing bleaching and, in some cases, even with dyeing, thus improving water and energy saving. Generally, alkali and detergent mixtures are used at temperatures around 95 °C - 98 °C. [34,35] Such a treatment is suited to continuous processing. In the process of Silk degumming the result can be ensured by

$$S = \frac{B - A}{B} \cdot 100 \left[\%\right]$$

Where,

S - Amount of removed sericin [%],

B - Mass of fibers before degumming [g], and

A - Mass of fibers after degumming [g].

3.2 Complications of Detergents

The detergent based silk degumming leads to the production of low quality of silk as detergent interfere with the mechanical properties of silk fibres. The fibroin, main part of silk protein provides tensile strength to silk fibres which is very sensitive to soap, alkali and alkaline protease. During the process of silk degumming often detergent interfere with weak interaction in fibroin protein and renders structural confirmation as a result silk losses it

mechanical parameter.[36] Another complication with detergent based silk degumming as chemicals used for process are not eco-friendly hence long term effects on the surrounding. Generally in the detergent based silk degumming soap derived from higher fatty acid has been used which still remains in the silk even after many washing. [37]

4. Microbial Protease in Silk Production

4.1 Need of Biological Detergents

The conventional detergents are not the choice for silk degumming as these chemical will interfere the basic physical and chemical properties of silk leading to the complication in the quality of silk. Though chemical based detergents have been used for long time but same time silk produced by these methods always has shorter shelf life and various other complications like less tensile strength, more hygroscopic etc. [38]

4.2 Various Sources for Proteases

Proteases cover the 60% of total enzyme market and amongst the most valuable commercial enzyme. Alkaline proteases hold a great potential for application in the detergent and leather industries and there is an ever increasing trend to develop environment friendly technologies. Plants, animals and microbes are the main sources for protease production. [39] The preferred sources of proteases are microbes because of their rapid growth and the ease with which they can be genetically manipulated to generate new enzymes with altered properties and are currently being utilized by the detergent industry eg. Serine proteases produced by Bacillus strains. Proteases from several bacteria have been purified and characterized. Genus *Pseudomonas* a gram-negative bacterium that predominantly produces alkaline proteolytic enzymes and the proteases has been purified. Fungal alkaline proteases are advantageous because of the ease of downstream processing to prepare a microbe-free enzyme at low cost production. [40]

Another way for the silk degumming various concentrations of dried latex of papaya fruit. The papaya's dried latex contains proteases which have been used in the various ranges 0%, 1%, 2%, 3%, and 4 % respectively to achieve efficient degumming. The degumming processes were run at the temperature ranges 55, 65, 75 and 85 and time 10, 20, 30 and 40 minutes. [41] The papaya fruit extract shown better results but again having same problem as the temperatures sensitive protease which we cannot run for higher temperature for longer time periods.

4.3 Complication with Conventional Microbial Protease

The protease and more specifically alkaline protease have shown numerious complication with the silk during the process of degumming. First of all the silk is highly sensitive to the alkali and alkaline protease which hampers it physical appearence. The Mechanical properties also get affected under the exposure of alkaline protease. Another complication with the conventional protease is their range of activity and stability in various pH and Temprature. Another significant complication especially in protease based silk deguming incorporation of chemicals in certian instants which also inactivates the conventional enzymes [42].

5. Thermostable Protease a Better Option

5.1 Need of Thermostable Protease

The optimal activity of conventional protease ranges 30-40`C which is not appropriate for compete degumming process. Even some time process has to run for more than 24 hour in at lower temperature which again causes deactivation of conventational enzyme. Often the degumming process runs at higher temperature which leads to denaturation of conventational proteases which are not thermostable. In some cases during the process a lots of chemical are required to complete the process and many circumstances presence of chemical also leads to the deactivation of enzyme and process subsequently. [43] To solve this process researchers are always in the hunt of enzyme which can hold on the higher temperature for long duration of time. Thermostable proteases are the choice for industrial process which runs at the higher temperature for long and silk degumming is one of that. The main advantage of thermostable protease is their range for activity 50-100`C and for long duration of time. The effect of various chemical and inhibitors is also quite less so in the future it will be choice for degumming. The availability of bacterial strain, isolation and purification of these proteases will further promote biological silk degumming without harming to environment. [44]

5.2 Various Sources of Thermostable Proteases

5.2.1 Bacterial Sources for Thermostable Protease

The proteases are generally classified into two broad categories (exopeptidases, that cleave off amino acids from the ends of the protein chain and endopeptidases, which cleave peptide bonds within the protein) are becoming major industrial enzymes, and constitute more than 65% of the world market [45] These protease enzymes have been extensively used in the food, pharmaceutical, leather and textile industries [46]. The applications will keep increasing in the future as will the need for stable biocatalysts capable of withstanding harsh conditions of operation.

The Bacilli provides 70% of protease hence the diverse sources has made these organisms the focus of attention in biotechnology [47]). Till date, however, few thermophilic Bacillus sp. that produce proteases have been isolated, the earliest isolate being Bacillus stereothermophillus [48] which is stable at 60`C. Another Bacillus sp. has produced a thermostable protease that has an optimum activity at 60`C [49], while a different Bacillus stereothermophillus sp. produced an alkaline and thermostable protease which is optimally active at 85 _C [50]. A species of Bacillus stereothermophillus TP26 that has been isolated produces an extra cellular protease having an optimum temperature of 75`C [51]. Enhancement of protease activity excreted from Bacillus stereothermophillus had also been possible using economical chemical additives in the proteolysis reactions involved in waste activated sludge [52]. In a chemically defined medium, thermophilic and alkaliphilic Bacillus sp. JB-99 was also reported to produce thermostable alkaline proteases [53] . Dominant producers of proteases in fact, are the microorganisms of the genera Pyrococcus, Thermococcus and Staphylothermus. Extremely thermostable serine proteases are produced by the hyperthermophilicarc haeum Desulfurococcus strain [54] , and thermostable metalloroteases are reported from a gram-negative thermophilic bacterium [55].

Table 4: Commercially available thermostable protease producing Bacterial strain

S No	Bacterial Strain	Range Temp	Range pH	Reference
1.	*Bacillus stearothermophilus*	80	9.0	Rahman et al. (1994)
2.	*Bacillus stereothermophillus*	60	9.0	Salleh et al. (1977)
3.	*Bacillus stereothermophillus TP26*	75	6-12	Johnevelsy and Naik (2001)
4.	*Bacillus licheniformis*	70	9.0	Manchini and Foretina (1998)
5.	*Bacillus brevis*	60	10.0	Banerjee et al. (1999)
6.	*Bacillus sp. JB-99*	80	6-12	Johnevelsy and Naik (2001)
7.	*Thermococcus aggreganes*	90	7.0	Klingberg et al. (1991)
8.	*Thermococcus litoralis*	85	8.5	Klingberg et al. (1991)
9.	*Thermococcus celer*	95	7.5	Klingberg et al. (1991)
10.	*Thermotoga maritema*	95	9.5	Klingberg et al. (1991)

5.2.2 Fungal Source for Thermostable Protease

Thermostable proteases are very efficient to improve the industrial processes in many fields. Many of thermostable extracellular proteases from the culture supernatant of the thermophilic fungus were purified to homogeneity by fractional ammonium sulfate precipitation, ion-exchange chromatography on DEAE-Sepharose, and Phenyl- Sepharose hydrophobic interaction chromatography. By SDSPAGE, the molecular mass of the two purified enzymes was estimated to be 33 kDa and 63 kDa, respectively. [56] The two proteases were found to be inhibited by PMSF, but not by iodoacetamide and EDTA. The 33 kDa protease (PRO33) exhibited maximal activity at pH 10.0 and the 63 kDa protease (PRO63) at pH 5.0. The optimum temperature for the two proteases was 65`C. The PRO32 had a Km value of 6.6 mM and a Vmax value of 10.31 µmol/l/min, and PRO63 17.6 mM and 9.08 µmol/l/min, with casein as substrate. They were hermostable at 60oC [57]. The protease activity of PRO33 and PRO63 remained at 67.2% and 17.31%, respectively, after incubation at 70oC for 1 h. The thermal stability of the two enzymes was significantly enhanced by Ca2+. The residual activity of PRO33 and PRO63 at 70oC after 60 min was approximately 88.59% and 39.2%, respectively, when kept in the buffer containing Ca2+. Hence proteases having following properties are quite efficient for the applicable for many biotechnological purposes [58,59].

6. Future Trends in Industrial Enzymology

6.1 Production and Purification of Thermostable Protease

In the last decades numbers of commercial uses have been established for thermostable amylases and roteases. In the brewing industry, starchy materials used as adjuncts must first be liquefied by the addition of thermostable amylases [61]. Thermostable amylases are used in desizing textiles to remove unwanted starch. They also may be used to aid in the clarification of fruit juices inthe manufacture of jelly and chocolate syrups [62,63]. Proteases are used in the leather industry, particularly in baiting hides to remove unwanted interfibrillar

material. In cheese making, the demand for milk-clotting enzymes besides rennet has increased. Other commercial outlets are available for thermostable hydrolases with suitable physicochemical properties.[64] Still many of process are optimizing to get pure enzyme in high yeild.

6.2 Optimization Study of Enzyme for Improved Silk Production

In the optimization of media compounds, Plackett-Burman designs are used as a screening method in order to select the variables that influence a system. However, they do not give an optimum value for each variable10 and further optimization is needed. Response surface methodology (RSM) has been widely used to evaluate and understand the interactions between different physiological and nutritional parameters [65]. It is an efficient mathematical approach widely applied in the optimization of the fermentation process and media component, e.g., production of enzymes, biomass, spore, and other metabolites12–15. RSM, which includes factorial design and regression analysis, can be used to help evaluate the effective factors and build models [66]. It can give information about the interaction between variables and can be used to select optimum conditions of variables for a desirable response and multiple responses at the same time. Finally, after model building and optimization, the predicted model is verified. In this investigation, an attempt has been made to improve silk degumming protease production from Bacillus subtilis C4 by statistical approaches using a Plackett-Burman design and RSM in submerged culture. The proteases obtained from the optimized medium using either shaken flasks or stirred tank reactors were also compared. [67]

Protein Engineering

Often the biological enzymes lack properties which are essential for an industrial application. Hence there is extensive search for new enzyme variants in organisms that grow in extreme conditions has been going on for more than 20 years but has resulted in relatively few successes. Sometimes a desired property, like extreme thermo stability, has been found but other problems have surfaced. The enzyme may not be functional in the desired temperature. It may also prove very difficult to overproduce the enzyme in a suitable host. Another option is to engineer a commercially available enzyme to be a better industrial catalyst. Two different approaches are presently available: a random method called directed evolution and a protein engineering method called rational design. [68]

Numerous enzymes have already been modified in order to function better in industrial processes. The protein engineering is the way by which following enzyme have achieved additional properties essential for industrial application are proteinases, lipases, cellulases, amylases and glucoamylases. In the recent year Xylanase has been refined, which needs to be stable in high temperature and active in physiological temperatures and pHs when used as feed additive and in alkaline conditions when it is used in bleaching in pulp and paper industry. One of the industrial production organisms of xylanase is *Trichoderma* fungus. [69] Its xylanase has been purified and crystallized. By designed mutagenesis its thermal stability has been increased about 2000 times at 70 ^{o}C and its pH-optimum shifted towards alkaline region by one pH-unit. The known structure of an enzyme is used to design and simulate mutations. The most successful strategies to improve the stability of the *Trichoderma* xylanase include the stabilization of the alpha-helix region and the N-terminus. [70] A progressive research is going on in order to refine the existing protease as per industrial applications.

Immobilization

In the present scenario the Immobilization is playing crucial role in Industrial level as we can reuse enzyme many times with the high efficient. This is a technique where enzyme makes fix on a support in order we can use in many cycles. An immobilized enzyme is is attached to an inert, insoluble material such as calcium alginate (produced by reacting a mixture of sodium alginate solution and enzyme solution with calcium chloride). This will provide increased resistance to changes in conditions such as pH or temperature.[71] Further also allows enzymes to be held in place throughout the reaction, following which they are easily separated from the products and may be used again - a far more efficient process and so is widely used in industry for enzyme catalysed reactions. The Immobilization is not only effective in the reuse of enzyme but also to provide resistance to enzyme towards chemicals, various pH and other biomolecules [72].

7. Conclusion

Research investigations in last decades have demonstrated potential application of extremophiles for various industrial applications with very high efficiency. The thermostable enzymes isolated from these organisms have just started providing conversions under conditions that are appropriate for industrial applications. The conditions required by these thermostable enzymes which bring about specific reactions not possible by chemical catalysts are still mild and environmentally benign, as compared to the temperatures and pressures required for chemical conversions. Thus, with the availability of thermostable enzymes a number of new applications in the future are likely. Although, believed to provide tremendous economical benefits, production of the enzymes to the level required by the industries has remained a challenge.

Protease based silk degumming has been shown higher efficiency, expressed over removal of sericin and energy save, achieved through lower process temperature. Milder treatment conditions under which the fibers were processed during enzymatic degumming prevented fibrillation and dusting i.e. fiber damage. Damage to the soap degummed fibers was enhanced in subsequent dyeing process, which was the reason of inability to spectrophotometrically measure dyestuff concentration in dye-bath. When dyeing silk fibers, method of degumming should be considered. After the degumming fibers still had lustrous, soft and smooth surface. Result of staining test with direct dyes was appeared pink, indicating that there was a small amount of sericin remaining. This process can be used instead of conventional degumming method with low cost and do not harm to environmental.

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