Biopolishing – An Overview

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In the recent years enzymes have found a variety of uses in textile applications. Popular uses are stone washing of denims and surface modification of cellulosic fabrics to improve their appearance and handle. The process of treating with cellulases is termed as bio- polishing. In case of denims one can get stone - wash effect without using pumice stones by using enzymes. Another advantage of using enzymes is that, these are environment friendly, since they are readily biodegradable. Besides, they will not leave chemical residue on the processed materials and the colour changes on the dyed goods are very less. Textiles goods treated with enzymes are free from surface hairiness and neps with much improved handle and flexibility. The fabric surface becomes smoothers and more lustrous. There is also a lower tendency to further pilling possibly due to the fact that there are less protruding fiber ends from the yarns after the enzymes application. Bio-finishing also called bio-polishing, is a finishing process applied to cellulose textiles that produces permanent effects by the use of enzymes. Bio-finishing removes protruding fibers and slubs from fabrics, Significantly reduces pilling, softens fabric hand and provides a smooth fabric appearance, especially for cotton fabrics and as a permanent for printing. Second rate articles can obtain the high value eye appeal of first rate ones. In denim processing; bio-finishing can reduce or eliminate abrasive stones and the aggressive chlorine chemistry, achieving the desired "worn" looks. Bio-finishing is not only useful for cotton but also for regenerated cellulose fabrics, especially for lyocell and microfiber articles. They have complex three dimensional structures composed of long chains of amino acid with molecular weights ranging from 10,000 to about 1, 50,000 and occasionally to more than 10,00,000. These naturally occurring molecules provide a high degree of catalytic specificity unmatched by man-made catalysts. The enzyme and substrate form a lock and key complex that requires the enzyme to have a specific molecular alignment in order to act as a catalyst. The lock and key theory of Emil Fischer was broadened by koshland jr to the induced-fit theory of the enzyme-substrate-complex. Chemical reactions catalysed by enzymes can typically be carried out, as is most usual in nature, under mild aqueous conditions without the need for high temperatures, extreme pH values or chemical solvents.

Enzymes are high molecular weight proteins produced by living organisms to catalyse the chemical reactions essential for the organism's survival. They have complex three-dimensional structures composed of long chains of amino acids with molecular weights ranging from 10,000 to about 1, 50,000 and occasionally to more than 10,00,000. These naturally occurring molecules provide a high degree of catalytic specificity unmatched by man-made catalysts. The enzyme and substrate form a 'lock and key' complex that requires the enzyme to have a specific molecular alignment in order to act as a catalyst. The lock and key theory of Emil Fischer was broadened by Koshland Jr. to the induced-fit theory of the enzyme-substrate-complex. Chemical reactions catalysed by enzymes can typically be carried out, as is most usual in nature, under mild aqueous conditions without the need for high temperatures, extreme pH values or chemical solvents. What a dream for every chemist.

Enzymes find commercial use in detergents, leather processing, baking, brewing cheese manufacture, fruit juice processing, dairy production, animal feed, wine making and textiles. A wide variety of different enzymes finds use in textile areas as shown in Table No.1. This article will focus on cellulases which are enzymes that catalyse the hydrolysis of the cellulose polymer. Except for the peroxidases, which are used to improve colour fastness, all the other types of enzymes are used for preparation processes and therefore not included in this discussion.

The names of the examples of textile-relevant enzymes follow the nomenclature of Duclaux from 1 898, characterising an enzyme by the end-syllable 'ase' added to the name of the substrate that is split, synthesised or otherwise catalysed. As with all catalysts, enzymes reduce the activation energy of a specific reaction. The discovery of large quantities of new enzyme systems afforded a more differentiated nomenclature, realised in 1964 by the International Union of Pure and Applied Chemistry (IUPAC) and the International Union for Biochemistry (IUB). In the new Enzyme Classification (EC) the first number refers to one of the six main groups and the following numbers to subgroups, for example EC 3.4.5.6, where 3 stands for hydrolases.

Wet-processing of cotton during preparation prior to dyeing involves the use of harsh chemicals. Also, chemicals are used during finishing imparting desirable attributes such as softness, durable press, and dimenstonal stability. Fortunately, in the past decade, commercially viable alternative methods for preparing and finishing cotton fiber substrates based on the use of enzymes have emerged. Such methods will ensure the supremacy of cotton over other fibers for decades to come. Enzymes are biological catalysts usually derived from a fungal or a bacterial source and consist of complex, three dimensional proteins that are composed of polypeptide chains (Etters et al., 1999).

Enzymes primarily function by promoting hydrolysis of specific substrates, a process by which water-insoluble material is converted to products that dissolve in water and can be washed away (Etters et al., 1999). Enzymatic hydrolysis has been successful in biofinishing and biopolishing of cotton. In biofinishing, dyed denim jeans are treated with solutions of cellulase under the proper conditions of temperature and pH. The surface of the treated fiber is hydrolyzed releasing dye in a random manner to produce the popular washed down or worn appearance. Enzymatic biofinishing has largely replaced the use of environmentally harsh pumice stones soaked in sodium hypochlorite or potassium permanganate that were previously used to obtain the worn effect. Cellulase is also highly effective in removing loose fibers from fabric surfaces, a process known as biopolishing. Not only does biopolishing produce a smooth fabric surface, it also assists a dyed fabric in retaining its color depth during laundering. This latter effect is achieved by incorporating cellulase in detergent formulations. Any fibers brought to the fabric surface by abrasion during launjering are hydrolyzed, which avoids the light scattering phenomenon that reduces color depth (Etters et al., 1999).

The term cellulase refers to a group of enzymes that act synergistically to hydrolyze cellulose. Cellulases perform a specific catalytic activity on the 1, 4- ~ - glucosidic bonds of the cellulose molecule. The hydrolysis of this bond cleaves the molecule into smaller parts that may be further reduced. Commercial cellulases, which are usually produced by submerged fermentation of Trichoderma reesei, are multicomponent enzyme

systems typically containing one or more exo-cellulase activities known as exo-cellobiohydrolases, multiple endo-glucanases, and betagluocosidases. Exo-cellulases act on cellulose polymer chain ends and produce primarily cellobiose. Endocellulases act randomly along the cellulose polymer chains breaking very long polymers into shorter chains. Betaglucosidases act on short, soluble oligosaccharides to produce primarily glucose (Karmakar, 1998; Kumar and Harnden, 1998). Synergism between the different components in the cellulase system has been documented, but detailed explanation of their mechanism and kinetics is not completely understood. The most widely proposed mechanism of hydrolysis of cellulose can be conveniently divided into the following stages (Lee and Fan, 1982): (a) transfer of enzyme molecules from the aqueous phase to the surface of cellulose molecules; (b) adsorption of the enzyme molecules onto the surface of cellulose resulting in the formation of an enzyme-substrate (E-S) complex; (c) transfer of molecules of water to the active sites of the E-S complex; (d) surface reaction between water and cellulose catalyzed by the E-S complex; and (e) transfer of the products of the reaction of cellobiose and glucose to the aqueous phase.

Table 1: Enzyme Treatments of Textiles Textile Use and Effects

Types of Enzyme	Textile Use and Effects
Cellulases	Biofinishing, biopolishing, anti-pilling, softness, smoothness, lustre improvement and stone-washed Effects on denim
Amylases	Standard procedure for the removal of starch warp size
Proteases	In household washing agents better removal of protein containing soil or stains. Anti-felting of wool, accompanied by high loss of weight, tear strength and of the typical handle, degumming of silk with the problem of silk fibroin damage
Lipases	In detergents for the hydrolysis of lipids
Pectinases	Hydrolysis of pectins, for example in cotton 'preparation' and retting of flax and hemp
Catalases	Catalyse the decomposition of hydrogen peroxide, important before reactive dyeing of printing of peroxide bleaching fabrics and yarn
Peroxidases	Used as an enzymatic rinse process after reactive dyeing, oxidative splitting of hydrolysed reactive dyes on the fibre and in the liquor, providing better wet fastness, decolourised waste water and potentially toxic decomposition compounds (aromatic nitro-compounds)
Ligninases	Removal of burrs and other plant compounds from raw wool
Collagenases	Removal of residual skin parts in wool
Esterases	In development: polyester finish, removal of 01 igomers
Nitrilases	In development: polyacrylonitrile preparation for better coloration

It is apparent from the steps in hydrolysis that the properties of the substrate, the multiple natures of the cellulase complex, and the mass transfer effects influence cellulose hydrolysis. A prerequisite for hydrolysis to occur is direct physical contact between the enzyme molecules and the surface of cellulose (Lee and Fan, 1982). Cellulase enzymes have a specific three-dimensional shape and their catalytic power depends on adsorption onto the surface of a substrate in lock-and-key fashion (Etters, 1998). A higher surface area enhances the accessibility of the enzyme molecules to the surface (Lee and Fan, 1982). Since cellulases are highly substrate specific in their action,

any changes in the structure and accessibility of the substrate has a profound influence on the kinetics of the hydrolysis reaction. Yarn type and fabric construction influence the hydrolysis rate (Karmakar, 1998). Key parameters for the cellulose substrate are accessible surface area, crystallinity, and pore dimensions. Changes in any of these factors, such as structural changes brought about by pre-treatments, influence the hydrolysis reaction. It has been reported that mercerized and raised fabrics are more accessible to enzymatic attack, because they have a more accessible structure (Cavaco-Paulo and Almeida, 1996a). Crystallinity is another structural feature regarded as important. The cellulolytic enzyme acts to a greater degree on the more accessible amorphous regions, so as crystallinity increases, cellulose becomes resistant to further hydrolysis (Lee and Fan, 1982). Milling of cellulose can increase the hydrolysis rates by reducing crystallinity or increasing the surface area (Busch Ie-Diller et al., 1998). Also, exo-cellulases assist in degradation of cellulose by disrupting the local crytalline cellulose structure, which makes the region more prone to attack by endo-glucanases (Karmakar, 1998).

Mass transfer effects playa decisive role in the kinetics of reaction. For example, enzyme diffusion plays an important role in the heterogeneous system of soluble enzyme and insoluble substrate. Kinetics of the reaction is therefore dependent on the diffusion of enzyme to and into the solid phase of the substrate and the diffusion of the reaction products out of the solid phase into the aqueous phase. Since cotton possesses only limited amorphous areas, diffusion into the interior of fibers is restricted, and the catalytic action of cellulase is confined to fiber surface (Heine and Hocker, 1995). Consistent with this theory, researchers have reported that the transfer of enzyme molecules to the surface is facilitated by agitation of the reaction mixture (Cavaco-Paulo and Almeida, 1996a), which helps enzyme adsorption and desorption, and aids in the removal of enzymatically loosened material from fiber surfaces (Buschle-Diller et al., 1998). For example, mechanical treatment in rotating drum washers and jets simultaneously with enzymatic hydrolysis is necessary in biopolishing to remove the fibers protruding from the surface (Cavaco-Paulo and Almeida, 1996 b). Where a two-step process is adopted with prior mechanical treatment followed by hydrolysis, a decrease in the efficiency of hydrolysis is observed (Zeyer et al., 1994). A smaller change in weight loss was also observed when a washing process followed cellulase treatment (Cavaco-Paulo et al., 1997). These observations confirm the importance of simultaneous mechanical agitation in the enzymatic hydrolysis process. In biofinishing of denim, mechanical action opens the outermost layers of the cellulosic crystal, which results in more of the cellulose being accessible to cellulase and in greater enzymatic removal of indigo. Use of ultrasonic energy in conjunction with mechanical agitation has also been reported to enhance efficiency of enzymatic treatment through reduced processing time, less concentration of enzyme, and better uniformity of enzymatic treatment (Yachmenev at al., 1998).

The products of reaction, namely cellobiose and glucose, profoundly influence the kinetics of-enzymatic hydrolysis of cellulose (Howell and Stuck, 1975; Lee and Fan, 1983; Chose and Das, 1971). Several researchers have reported that the accumulation of sugars inhibits the hydrolytic action of the cellulolytic enzyme. Chose and Das (1971) was among the first to report that cellulase was competitively inhibited by cellobiose.

Huang (1978) studied the enzymatic hydrolysis of insoluble amorphous cellulose by T. reesei and modeled the reaction by taking into account the fast adsorption of enzyme followed by slow reaction and subsequent product inhibition. Howell and Stuck (1975) developed a product inhibition on the assumption that noncompetitive inhibition by cellobiose dominates the reaction kinetics for the T. reesei system. Applicability of the model was supported by agreement of predicted and experimental reaction progress when cellobiose was added to the reaction mixture or when product was continuously removed from the membrane reactor. A related work argued that the products of hydrolysis decreased the hydrolysis rate by facilitating a loss of enzymatic activity (Howell and Mangat, 1978). Other researchers have also observed a drastic decline in hydrolysis rate during the initial hours of hydrolysis (Lee and Fan, 1983). Two factors were identified as responsible for causing the reduction in the hydrolysis rate. The first factor was a structural transformation of cellulose into a less digestible form as evidenced by changes in the crystallinity index and surface area. The second factor was product inhibition. Effects of crystallinity of cellulose, thermal deactivation, and cellobiose and glucose inhibition on the hydrolysis rate of pure cellulose have been quantitatively investigated and an empirical rate expression has been developed (Ohmine et al., 1983). An excellent literature summary of cellulase inhibition classified according to enzyme, substrate, inhibitor, and nature of inhibition has been published (Holtzapple et al., 1990). Factors affecting product inhibition include the nature of substrate, substrate concentration, enzyme concentration, and composition of the cellulase multi-component system. Among these factors, the most important one is the enzyme/substrate ratio. Different product inhibition patterns may be observed depending on the enzyme concentration and variation in the range In substrate concentration (Gusakov and Sinitsyn, 1992).

It is apparent from the preceding discussion that a lot of effort has been expended on developing kinetic models to explain the complex phenomenon of enzymatic hydrolysis, but these studies have been conducted using variations of the Michaelis-Menten initial velocity kinetics. The MichaelisMenten equation developed for enzymatic reactions in solution may not be valid since cellulose is an insoluble substrate. Also, due to the complexity of the hydrolysis process most models have tended to focus on one specific aspect of the hydrolysis process to the exclusion of other simultaneously occurring phenomenon. To concurrently take into account the various changes taking place during hydrolysis, an alternative approach is the application of empirical equations to model experimental data. An empirical rate expression was developed for several kinds of non-textile cellulosic substrates (Ohmine et al., 1983; Ooshima et al., 1982; Kurakake et al., 1995). Unfortunately, those equations do not hold very well for cotton fiber substrates.

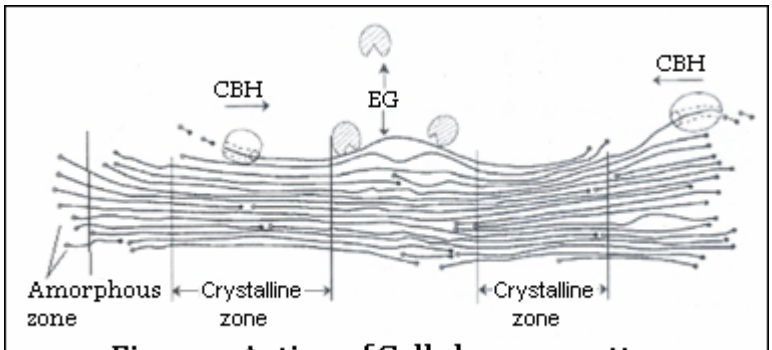

Figure 1: Action of Cellulases on cotton

Cellulases

Cellulases are high molecular colloidal protein bio-catalyst in metabolite form. Industrial

cellulases represent complex of a number of cellulases, cellobiase and related enzymes of completely non uniform composition in a molecular weight range of 10,000 to 4 million.

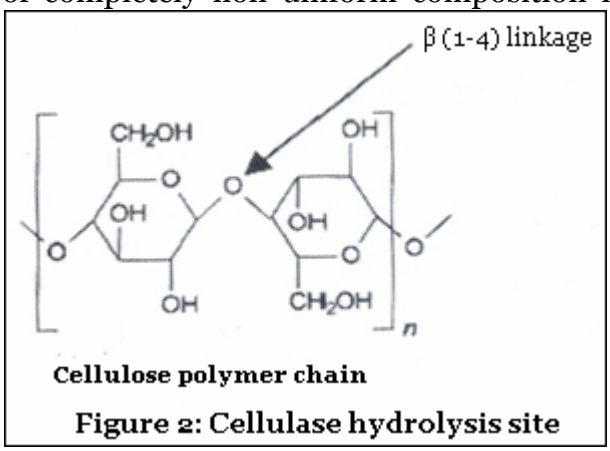

Figure 2: Cellulase hydrolysis site

Enzymes or cellulases have a protein like structure with primary, secondary, tertiary and quaternary structures and that are susceptible to degradation due to temperature, ionizing radiation, light, acids, alkali, and biological effect factors. Cellulases are capable of breaking the 1,4-β- glucoside bond of cellulose randomly. When cotton fabric is treated with a cellulase solution under optimum condition: Cellulase hydrolyse cellulose by reaching to the 1,4-β- glucoside bond of the cellulose molecule.

As a result of which the fabric surface becomes smooth with the loss of surface fibres and the hand becomes soft. There is also loss in strength proportional to the amount of weight reduction.

There are mainly three types of cellulases:

1. Acid stable (more effective in pH range of 4.5 - 5)
2. Neutral stable (effective at pH 7)
3. Alkaline stable (not used widely)

Action of Cellulase Enzymes on Cellulose

Enzymes that hydrolyse cellulose are found in nature in both Trichoderma and Humicola fungi. The β(1-4) linkages between adjacent repeat units in the cellulose polymer chain are the sites that are vulnerable to catalytic hydrorysis by cellulases (Figure 1 and 2). These enzymes are thus able to provide a food source for the organisms by producing glucose from cellulose. Industrial production of cellulases involves large scale growth of fungal populations, followed by extraction of the enzyme. The extracted enzymes contain multiple components that work together to yield glucose from a cellulose polymer chain. At least four components have been identified as being important in providing efficient glucose production. Endo-glucanases hydrolyse cellulose at random locations along the polymer chain. Beta-

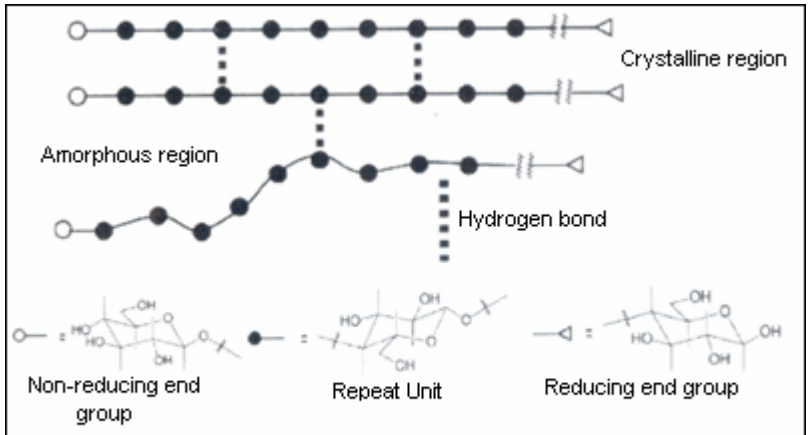

Figure 3: Schematic representation of cellulose fibre internal structure

glucanases hydrolyse cellulose polymers from the non reducing end producing glucose and leaving a polymer chain with one less repeat unit. Cello-biohydrolases produce cellobiose (the glucose dimer) from polymer chains, and cellobiases convert cellobiose into glucose. These components and their points of attack on cellulose polymer chains are shown schematically in Figure 1, 2 and 3.

Numerous studies of the mechanism of cellulase interactions with cellulose have been reported. The generally accepted mechanism is adsorption of the endoglucanase, beta-glucanase or cellobiohydrolase components onto the fibre surface followed by complex formation with the cellulose polymer chain and water. After hydrolysis of the β(1-4) bond, the enzyme desorbs and is available for further adsorption and reaction. Endo-glucanase effectively opens up more of the fibre structure to attack by beta-glucanase and cellobiohydrolase. These two components in turn produce water-soluble fragments from the exposed area. This synergistic action allows for rapid reduction in fibre strength.

Chemistry of Biopolishing

More than with other chemical reactions, the enzyme catalysed hydrolysis of cellulose is strongly influenced by factors such as pH, temperature, time and agitation. The optimal pH for a particular cellulase depends upon its origin. Trichoderma-based products (sometimes called 'acid cellulases') work best at pH 4.5-6, whereas cellulases from Humicola (often called 'neutral cellulases') are more effective at pH 6-6.5. The reaction temperature is also critical since at low temperatures, the reaction rate is slower than desired, but very high temperatures can deactivate the enzyme by providing enough energy to alter its molecular alignments and thereby destroy its catalytic ability. Since enzymes are true catalysts and are not consumed during the chemical reaction, the hydrolysis reaction will continue until either the reaction conditions change or the cellulose is physically removed from the reaction mixture. Mechanical agitation is important in order for the hydrolysis reaction to proceed efficiently. Recent work has demonstrated that the kinetics of the reaction is controlled by mass transfer effects. The adsorption- desorption mechanism of enzyme action depends on agitation to remove hydrolysis by-products and expose new fibre areas to attack. The action of cellulose on cellulose is depicted in Figures 4, 5 and 6.

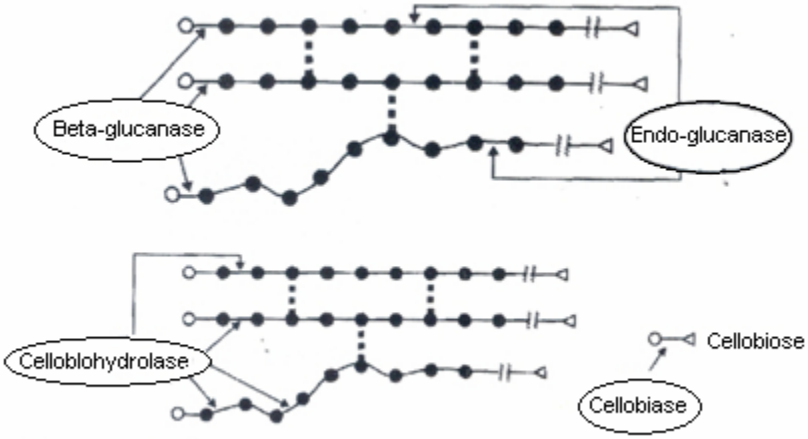

Figure 4: Action of cellulase components on cellulose

Because the enzyme's catalytic action is not reduced during the reaction, an effective method of ending the hydrolysis must be employed to prevent excessive fibre loss. Since the molecule's physical alignments are crucial to its catalytic ability, procedures that alter the cellulase

molecule's internal structure can be used to deactivate the catalysis and halt the hydrolysis. High temperatures (> 70°C or 160°F for at least 20 minimum or short drying at 120°C or 248°F), high pH (>10) and high electrolyte content as well as enzyme poisons can serve to terminate the reaction by distorting the enzyme's molecular shape.

Recent developments in enzyme manufacturing have led to commercial products that contain a preponderance of one cellulase component. These 'mono-component' enzymes are produced from modified Humicola strains and are primarily endo-glucanases active at pH 7-7.5 and are referred to as 'alkaline cellulases'.

Cellulose has been used on a large scale for years in medicine analysis, food chemistry and other industries. Cola strains and are primarily endo-glucanases active at pH 7 7.5 and are referred to as 'alkaline cellulases'.

Enzyme Inactivation

To prevent any damage of the fabric after the finishing operation it is very essential that the reaction be terminated at the end of treatment by enzyme inactivation. If the enzyme is not inactivated entirely then at the end of the reaction fibres get damaged and even extreme cases total destruction of the material may result. The enzyme inactivation is therefore of great importance from the technical point of view.

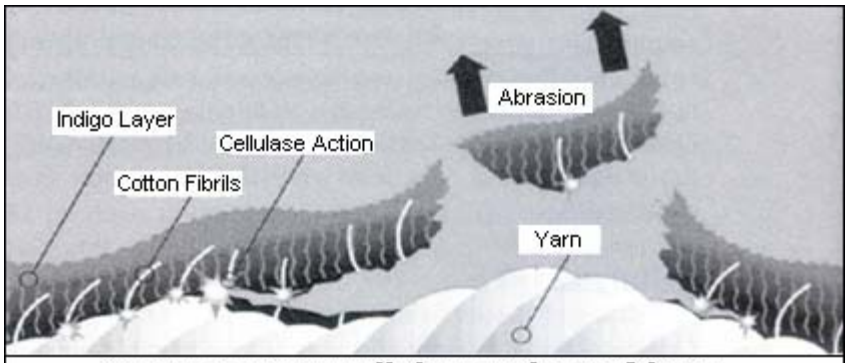

Figure 5: Action cellulase on denim fabrics

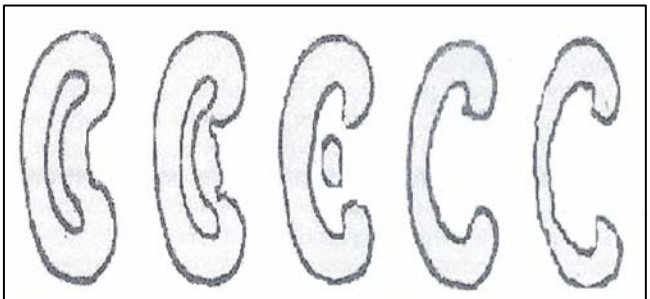
Figure 6 : Changes in morphology of cotton fibre during cellulase treatment (Kassenback, 1970)

There are two distinct process of termination of enzyme:

1. Hot treatment at 80°C for 20 minutes.
2. By raising the pH to 11-12.

Advantages of Biopolishing

- Hairiness, fluffs and pills are removed.
- Material sticking (the burr effect) is prevented.
- Improved handle.
- Achievement of surface smoothness and a clear
- structural appearance.
- Improved lustre.
- Material texture relaxation.
- Increased flexibility and therefore a soft handle, even with over end-products and mercerized fabric.

- Improved sewability.
- Fast to washing, low pilling tendency, no napping in use, or during care operation.
- Stone wash effect without pumice stone and dyestuff destroying chemicals.
- Poor quality, uneven, napped, knoppy material surface (ie.) typical second quality goods are converted into elegant, lustrous, soft, top quality with a fine, high quality surface appearance.

The surface morphology of cellulase treated cellulose is shown in Figure 7.

Disadvantages of Biopolishing

- Loss in weight.
- Loss in strength.

Troubleshooting for Biopolishing

As mechanical agitation important to affect the biofinishing, only selected processes and machines can be used, for example tubular fabric preferably cut to open width and treated in open width washer. In the rope from the loosened fibre particles are filtered out by the fabric and cannot easily be removed. The pad-batch process, jig or package dyeing machines are not effective in biofinishing.

Not all cellulase enzymes give identical results, even with similar equipment. Cellulases derived from Trichoderma typically are the most aggressive in their action, whereas mono-component endo-glucanases often require the most mechanical action to achieve the desired effects. Slow deactivation of the cellulases during transport and storage can adversely affect the reproducibility of the resulting effects. If cotton is not washed carefully before bio-finishing, secondary fibre compounds as residual: biocides can deactivate the cellulases. The same is true for natural or synthetic tannic acids, and resist or fastness improving agents for wool or nylon in cellulose fibre blends. Deactivation of cellulases after the desired effects have been achieved is very important. If the enzyme is not completely removed from the fabric, or is not effectively deactivated, the hydrolysis reaction will continue, although at a slower rate. As vary large molecules, cellulases cannot diffuse into the crystalline parts of the cellulose fibers. They react on the fibre surface, so fibre damage takes time. But have place to weaken the affected fabrics or garment, leading to customer complaints and returns. Undesirable deactive may be caused by high temperature and time, for example, caused by high transport and storage and also by enzyme poisons such as certain surfactants (especially cationic ones), formaldehyde-containing products or heavy metal ions. An activation effect on cellulases was reported by Nicolai and co-workers. Alkaline pretreatment, low concentrations of selected non-ionic surfactants, polycarboxylic acids and enhance the bio-finishing of celluloses. The use of pH buffers during the hydrolysis reaction is strongly recommended, especially when abrading denim fabrics. Cellulase enzymes have very narrow pH ranges of effectiveness and denim fabrics can have significant quantities of residual alkali from the indigo dyeing process. Buffers are required to maintain the appropriate reaction conditions for maximum enzymes effectiveness. Because the effect of processing auxiliaries on predict, it is important to evaluate any changes in processing

formulas carefully by conducting small scale trials before making significant changes in production procedures.

Removal of protruding fibres from garment surface using cellulase enzymes is called bio-polishing they act upon the short fibres protruding from fabric surface and make the fibers weak which are easily removed during washing. This process imparts soft and smooth feel and reduce fuzz or pilling tendency. This process is applicable to garment made of cotton and its blends. Two kinds of cellulases are commercially available, acid cellulases, which have activity in luases, which have activity in acidic medium in pH range of 4.5 - 5.5 and natural cellulases, which have activity in pH range of 5.5 - 8.0. Both these types are active in the range of 45°C to 60°C.

Conclusions

Biopolishing is an enzymatic process for finishing of cellulosic materials such as cotton, linen, viscose, ramie and their blends with synthetic fibres. It is a process that removes fuzz and eliminates pilling in cellulosic garments. The enzyme performs a controlled hydrolysis of the cellulosic fibres in order to modify the fabric surface and giving them fuzzy texture. Repeated usage make yarns breakage and their ends resulting in pilling and make a new garment look old. Biopolishing keeps the garments looking new even after repeated washes. Biopolishing removes protruding fibres to improve the texture and appearance of fabrics and also improves colour, feel, luster and drape. Its effects are permanent, without involving chemical coating of the fibre. At the same time, the treatment results in certain adverse effects like loss in weight and strength. All the above effects are influenced by number of factors like concentration of cellulase, pretreatments given to the fabrics and process parameters used at the time of treatment. Hairiness on the linen surface causes scratchiness and uncomfortable in serviceability. Softness and appeal of linen fabric can be greatly improved by biopolishing.

Acknowledgment:
The authors wish to express their sincere thanks to Prof. Dr. P.V, Kadole, Principal and Prof. Dr. A.I. Wasif of Textile & Engineering Institute, India for their inspiration and moral support for carrying out this research work.

This article was originally published in "The Textile Review", June, 2012.