

# Enzymes: A revaluation in textile processing

by Muhammad Ayaz Shaikh, Assistant Professor, College of Textile Engineering, SFDAC.

## Abstract

The use of enzymes in the textile chemical processing is rapidly gaining globally recognition because of their non-toxic and eco-friendly characteristics with the increasingly important requirements for textile manufacturers to reduce pollution in textile production. Enzymes sources, activity, specificity, reaction, mechanism and thermodynamics, function of textile processing with enzymes, major enzymatic applications in textile wet processing and promising areas of enzyme applications in textile processing are discussed. The aim is to provide the textile technologist with an understanding of enzymes and their use with textile materials.

## Enzymes are proteins

Enzymes are generally globular proteins and like other proteins consist of long linear chains of amino acids that fold to produce a three-dimensional product. Each unique amino acid sequence produces a specific structure, which has unique properties. Individual protein chains may sometimes group together to form a protein complex.

## Biocatalyst

Enzymes are biocatalysts, and by their mere presence, and without being consumed in the process, enzymes can speed up chemical processes that would otherwise run very slowly. After the reaction is complete, the enzyme is released again, ready to start another reaction. Most of the biocatalyst have limited stability and over a period of time they lose their activity and are not stable again. Usually most enzymes are used only once and discarded after their catalytic action.

## Nomenclature

The International Union of Biochemistry and Molecular Biology have developed a nomenclature for enzymes, the EC numbers where each enzyme is described by a sequence of four numbers preceded by "EC". The first number broadly classifies the enzyme based on its mechanism.

The top-level classification is

- EC 1 Oxidoreductases: catalyze oxidation/reduction reactions.
- EC 2 Transferases: transfer a functional group.
- EC 3 Hydrolases: catalyze the hydrolysis of various bonds.
- EC 4 Lyases: cleave various bonds by means other than hydrolysis and oxidation.
- EC 5 Isomerases: catalyze isomerization changes within a single molecule.
- EC 6 Ligases: join two molecules with covalent bonds.

At present, more than 2,000 enzymes have been isolated and characterized. Among them about 50 microbial enzymes have industrial applications.

There is a large number of microorganisms which produce a variety of enzymes. Microorganisms producing enzymes of textile important are listed Table 1.

## Activity

The activities of enzymes are determined by their three-dimensional structure. Most enzymes can be denatured, which disrupt the

Table-1

Micro organisms	Enzymes
<b>1. Bacteria</b>	
Bacillus subtilis	Amylase
B. coagulans	$\alpha$ amylase
B.licheniformis	$\alpha$ amylase, protease
<b>2. Fungi</b>	
A. niger	Amylases, protease, pectinase, glucose oxidase
A. oryzae	Amylases, lipase, protease
Candela lipolytica	Lipase
P. notatum	Glucose oxidase
Rhizopus sp	Lipase
Trichoderma reesei	Cellulase
T. viride	Cellulase
Ascomycetes	$\alpha$ amylase
Basidiomycetes	$\alpha$ - amylase
Aspergillus sp	Pectinase, lipase

three-dimensional structure of the protein. Denaturation may be reversible or irreversible depending on the enzyme.

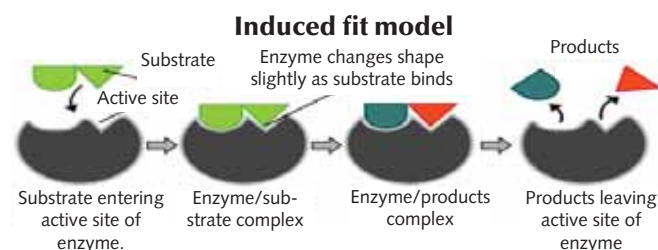
There are two proposed models of enzyme substrate complex formation.

### 1. Lock-and-key Model or Template

In 1894 Emil Fischer provided the lock-and-key model assuming that the active site is a perfect fit for a specific substrate and that once the substrate binds to the enzyme no further modification is necessary. It is a simplistic model.

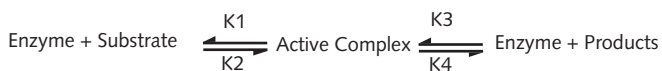
### 2. Induced fit Model or Koshland Model

In 1958 Daniel Koshland suggested a modification to the lock and key model. Instead of flexible structures, the active site is continually reshaped by interactions with the substrate as the substrate interacts with the enzyme. As a result, the substrate does not simply bind to a rigid active site; the amino acid side chains which make up the active site are molded into the precise positions that enable the enzyme to perform its catalytic function. In some cases, such as glycosidases, the substrate molecule also changes shape slightly as it enters the active site. The active site continues to change until the substrate is completely bound, at which point the final shape and charge is determined. The product is usually unstable in the active site due to steric hindrances that force it to be released and return the enzyme to its initial unbound state.



## Enzymatic reactions

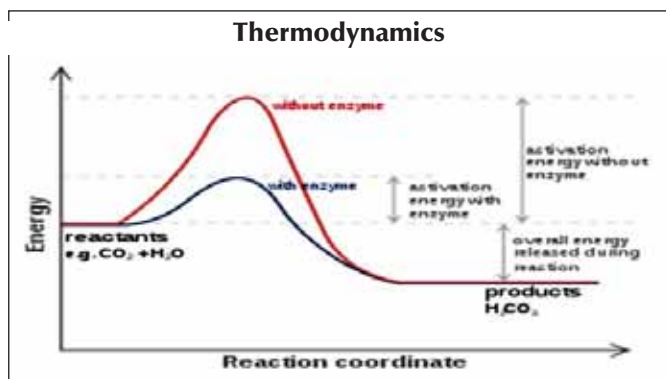
Victor Henri elaborated enzyme reactions in two stages. In the first, the substrate binds reversibly to the enzyme, forming the enzyme-substrate complex. This is sometimes called the Michaelis complex. The enzyme then catalyzes the chemical step in the reaction and releases the product.



## Mechanisms

Enzymes can act in several ways, all of which lower  $\Delta G^\ddagger$ :

- ❖ Lowering the activation energy by creating an environment in which the transition state is stabilized.
- ❖ Lowering the energy of the transition state, but without distorting the substrate, by creating an environment with the opposite charge distribution to that of the transition state.
- ❖ Providing an alternative pathway. For example, temporarily reacting with the substrate to form an intermediate ES complex, this is not possible without enzyme.
- ❖ Reducing the reaction by bringing substrates together in the correct orientation to react.
- ❖ Reactions speed up with increase in temperatures. However, the enzyme's shape deteriorates on overheating and only when the temperature comes back to normal does the enzyme regain its shape. Some enzymes like thermolabile enzymes work best at low temperatures.



Enzymes like all catalysts do not alter the position of the chemical equilibrium of the reaction. They do not alter the equilibrium itself, but only the speed at which it is reached. Usually, in the presence of an enzyme, the reaction runs in the same direction as it would without the enzyme, just more quickly. However, in the absence of the enzyme, other possible uncatalyzed, spontaneous reactions might lead to different products, because in those conditions this different product is formed faster.

## Enzymes in textile

Today enzymes have become an integral part of the textile processing. There are two well-established enzyme applications in the textile industry. Firstly, in the preparatory finishing area **amylases** are commonly used for **desizing process** and secondly, in the finishing area **cellulases** are used for **softening, bio-stoning and reducing of pilling propensity** for cotton goods.

However, there is little known about potential enzyme usage in other textile areas. At present, applications of pectinases, lipases, proteases, catalases, xylanases etc., are used in textile processing. There are various applications which entail enzymes included fading of denim and non-denim, bio-scouring, bio-polishing, wool finishing,

peroxide removal, decolourization of dyestuff, etc. Now the use of biocatalyst has become state of the art in the textile industry. Research and development in this sector is primarily concentrating on:

- ❖ Optimizing and making routine the use of technical enzymes in processes that are already established in the textile industry today.
- ❖ Replacing established conventional processes with the aid of new types of enzymes, particularly from extremophile micro-organisms, under stringent conditions.
- ❖ Preparing enzyme-compatible dyestuff formulations, textile auxiliary agents and chemical mixtures.
- ❖ Producing new or improved textile product properties by enzymatic treatment.
- ❖ Providing biotechnological dyes and textile auxiliary agents, which are suitable for industrial use, and can possibly be synthesized in-situ.

Most of the textile enzymes are those that catalyze the digestion or hydrolysis of certain large organic molecules like starch, cellulose, and protein. The enzymes actually attack these complex molecules, accelerating their digestion and yielding simpler substances. Since this process of digestion is referred to as hydrolysis, the enzymes that catalyze the process are considered to be hydrolyzing enzymes or hydrolases.

### The hydrolyzing enzymes include:

1. Amylases, which catalyze the digestion of starch into small segments of multiple sugars and into individual soluble sugars.
2. Proteases or proteinase, which split up proteins into their component amino acid building blocks.
3. Lipases, which split up animal and vegetable fats and oils into their component part glycerol and fatty acids.
4. Cellulases (of various types) which break down the complex molecule of cellulose into more digestible components of single and multiple sugars.
5. Beta-glucanase or gumase, which digests one type of vegetable gum into sugars and / or dextrins.
6. Pectinase which digests pectin and similar carbohydrates of plant origin.

## Amylase

All amylases are glycoside hydrolases and act on  $\alpha$ -1, 4-glycosidic bonds that hydrolyse starch down into sugar. Amylase is present in human saliva; the pancreas also makes amylase. Plants and some bacteria also produce amylase.

Specific amylase proteins are designated by different Greek letters. The  $\alpha$ -amylases (EC 3.2.1.2) are calcium metalloenzymes, completely unable to function in the absence of calcium. By acting at random locations along the starch chain,  $\alpha$ -amylase breaks down long-chain carbohydrates, ultimately yielding maltotriose and maltose from amylose, or maltose, glucose and limit dextrin from amylopectin.  $\alpha$ -amylase tends to be faster-acting than  $\beta$ -amylase as it can act anywhere on the substrate.

$\beta$ -amylase (EC 3.2.1.2) is also synthesized by bacteria, fungi, and plants. Working from the non-reducing end,  $\beta$ -amylase catalyzes the hydrolysis of the second  $\alpha$ -1, 4 glycosidic bond, cleaving off two glucose units (maltose) at a time.

In addition to cleaving the last  $\alpha$ -1, 4 glycosidic linkages at the nonreducing end of amylose and amylopectin, yielding glucose,  $\gamma$ -amylase (EC 3.2.1.3) cleaves  $\alpha$ -1, 6 glycosidic linkages. Unlike the other forms of amylase,  $\gamma$ -amylase is most efficient in acidic environments and has an optimum pH of 3.

Starch is used as a sizing agent in textile comprising of linear chained amylose and branched chain amylopectin. The desizing process was carried out by treating the fabric with chemicals such as acids, alkali or oxidising agents.

The amylose is bioconverted to 100% by the alpha- amylase into glucose whereas the amylopectin is converted to 50% into glucose and maltose. Bio desizing is preferred due to their high efficiency and specific action. Amylases bring about complete removal of the size without any harmful effects on the fabric besides eco friendly behavior.

### Pectinase

Pectinase (EC 3.2.1.15) is a general term for enzymes such as pectolyase, pectozyme and polygalacturonase. Pectinases hydrolyse pectin, a polysaccharide substrate that is found in the cell walls of plants into galacturonic acid and small sugars. Commercially available pectinases contain only very little cellulases and fiber damage should be limited as cellulose itself is not targeted.

Pectinases are reported from various microbial sources. Fungal pectinases have been extracted from *Aspergillus niger*, *Penicillium frequentans*, *Sclerotium rolfsii*, and *Rhizoctonia solani*. However these enzymes are optimally active in acidic conditions. Alkaline active pectinases have been obtained from *Penicillium italicum* and *Aspergillus sp.* Pectinases have an optimum temperature and pH at which they are most active. For example, a commercial pectinase might typically be activated at 45 to 55 °C and work well at pH of 4.5 to 5.5. If pectinase is boiled it is denatured making it harder to connect with the pectin at the active site.

Today, highly alkaline chemicals caustic soda is used for scouring. These chemicals not only remove the non-cellulosic impurities from the cotton, but also attack the cellulose leading to heavy strength loss and weight loss in the fabric. Furthermore, using these hazardous chemicals result in high COD, BOD and TDS in the waste water.

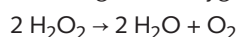
Recently a new enzymatic scouring process known as 'Bio-Scouring' is used in textile wet-processing with which all non-cellulosic components from native cotton are removed. After this Bio-Scouring process, the cotton has an intact cellulose structure, with lower weight loss and strength loss. The fabric gives better wetting and penetration properties, making subsequent bleach process easy and resultantly giving much better dye uptake. It also reduces environmental burden by reducing waste water treatment.

Enzymatic scouring process can be applied to cellulosic fibres and their blends (for both woven and knitted goods) in continuous and discontinuous processes. When enzymatic desizing is applied, it can be combined with enzymatic scouring. The process can be applied using jet, overflow, winch, pad-batch, pad-steam and pad-roll equipment.

### Catalase

Catalase (EC 1.11.1.6) is a tetramer of four polypeptide chains, each over 500 amino acids long. It contains four porphyrin heme groups that allow the enzyme to react with the hydrogen peroxide. Catalase has one of the highest turnover numbers of all enzymes; one molecule of catalase can convert millions of molecules of hydrogen peroxide to water and oxygen per second. The optimum pH for human catalase is approximately 7, and has a fairly broad maximum (the rate of reaction does not change appreciably at pH between 6.8 and 7.5). The pH optimum for other catalases varies between 4 and 11 depending on the species. The optimum temperature also varies by species. Catalase is a common enzyme found in nearly all living organisms. Catalase is also universal among plants, and many fungi are also high producers of the enzyme.

Natural fabrics such as cotton are normally bleached with hydrogen peroxide before dyeing. Catalase enzyme is used to break down hydrogen peroxide bleaching liquor into water molecules and less reactive gaseous oxygen.



Compared with the traditional clean-up methods, the enzymatic process results in cleaner waste water or reduced water consumption, a reduction of energy and time. Reuse of the bleaching liquor after hydrogen peroxide bleaching is already possible today by using the enzyme catalase after bleaching. This enzyme destroys excess hydrogen peroxide, making use of the bleaching liquor for other finishing stages possible.

### Cellulase

Cellulase, 1, 4-(1,3;1,4)-β-D-Glucan-4-glucanohydrolase (EC 3.2.1.4) is a linear polysaccharide of glucose residues connected by β-1,4 linkages. Cellulase refers to a class of enzymes although cellulases are distributed throughout the biosphere; they are most manifest in fungi and microbial sources. Cellulases are produced chiefly by fungi, bacteria, and protozoans that catalyze the cellulolysis of cellulose. However, there are also cellulases produced by other types of organisms such as plants and animals. There are several different kinds of cellulases which differ structurally and mechanistically. *Trichoderma reesei* secretes cellulase in high amount, therefore this fungus is used for commercial production of cellulase.

There are three types of reaction catalyzed by cellulases:

1. Breakage of the non-covalent interactions present in the crystalline structure of cellulose (endo-cellulase).
2. Hydrolysis of the individual cellulose fibers to break it into smaller sugars (exocellulase).
3. Hydrolysis of disaccharides and tetrasaccharides into glucose (beta-glucosidase).

The commercially available cellulases are a mixture of enzymes viz., Endogluconases, Exogluconases and Cellobiases. Endogluconases are subclass of cellulase enzymes which randomly attack the cellulose and hydrolyze the 1-4 glucosidic linkage of cellobiose chain. Exogluconases of cello-biohydrolases are again subclass of cellulose enzyme which hydrolyses 1-4 glucosidic linkage of cellulose to release cellobiose from the cellulose chain and Cellobiases are enzymes which hydrolyse cellobiose into soluble glucose units. All these three enzymes act synergistically on cellulose to hydrolyse them.

### Protease

A protease enzyme breaks down proteins. It conducts proteolysis, that is, begins protein catabolism by hydrolysis of the peptide bonds that link amino acids together in the polypeptide chain forming the protein. Proteases work best in acidic conditions. Proteases, also known as proteinases or proteolytic enzymes occur naturally in all organisms and belong to hydrolase class of enzymes, classified based on the source from which it is extracted, optimum temperature of activity. Proteases precisely act on peptide bonds formed by specific amino acids to hydrolyze them.

Commercial proteases are available, which can work in different range of pH and temperature. Trypsin (pancreatic), Papain based and alkaline proteases find industrial applications in textiles. The modified proteolytic enzyme enables the reaction of the enzyme with wool to be controlled, so that less degradation of the wool occurs than in similar treatments with the native protease. An anti-felting effect has been achieved without any significant weight loss being caused by the modified protease during the treatment. This novel enzymatic process leads to environmentally friendly production of machine washable wool.

Proteases are the most widely used enzymes in the detergent industry. They remove protein stains such as grass, blood, egg and human sweat. These organic stains have a tendency to adhere strongly to textile fibres. Proteases hydrolyse proteins and break them down into more soluble polypeptides or free amino acids. As

a result of the combined effect of surfactants and enzymes, stubborn stains can be removed from fibres.

### Laccase

Laccases (EC 1.10.3.2) are copper-containing oxidase enzymes that are found in many plants, fungi, and microorganisms. The use of lignin degrading white-rot fungi has attracted increasing scientific attention as these organisms are able to degrade a wide range of recalcitrant organic compounds such as polycyclic aromatic hydrocarbons, chlorophenol, and various azo, heterocyclic and polymeric dyes. The major enzymes associated with the lignin degradation are laccase, lignin peroxidase, and manganese peroxidase. These enzymes can be used for textile dyeing/finishing, and many other industrial, environmental uses.

In textile dyeing large amounts of dyestuffs are used. The discharge effluent has high COD, BOD, suspended solids and intense colour due to the extensive use of dyes. This type of water must be treated before discharging it into the environment. It was found that the fungi *Trametes Modesta* laccase showed the highest potential to transform the textile dyes into colourless products. The rate of the laccase catalyzed decolourization of the dyes increases with the increase in temperature up to certain degree above which the dye decolourization decreases or does not take place at all. The optimum pH for laccase catalyzed decolourization depends on the type of the dye used. Textile dyestuffs with different structures are decolourized at different rates.

Another study carried out by E. Abadulla et al, has shown that the enzymes *Pleurotus ostreatus*, *Schizophyllum Commune*, *Sclerodium Rolfsii*, *Trametes Villosa*, and *Myceliophthora Thermiphilia* efficiently decolourized a variety of structurally different dyes. This study also shows that the rate of reaction depends on the structure of the dye and the enzyme.

### Lipase

A lipase (EC 3.1.1.3) is a water-soluble enzyme, a subclass of the esterases that catalyzes the hydrolysis of ester bonds in water-insoluble, lipid substrates. It is primarily produced in the pancreas but is also produced in the mouth and stomach. Most people produce sufficient amounts of pancreatic lipase. Lipases from fungi and bacteria serve important roles in human practices as ancient as yogurt and cheese fermentation. However, lipases are also being exploited as cheap and versatile catalysts to degrade lipids in more modern applications.

Though enzymes can easily digest protein stains, oily and fatty stains have always been troublesome to remove. The trend towards lower washing temperatures has made the removal of grease spots an even bigger problem. This applies particularly to materials made up of a blend of cotton and polyester. The lipase is capable of removing fatty stains such as fats, butter, salad oil, sauces and the tough stains on collars and cuffs.

### Glucose oxidase

The glucose oxidase enzyme (GOx) (EC 1.1.3.4) is a dimeric protein. It is naturally found in honey. Commercially, it is often extracted from *Aspergillus niger*.

At university of Auburn (USA) glucose oxidase was used for bleaching. The result showed whiteness index 15-20 degree improvement with low strength loss. Conventional preparation of cotton requires high amounts of alkaline chemicals and consequently, huge quantities of rinse water are generated.

An alternative to this process is to use a combination of suitable enzyme systems. Amyloglucosidases, Pectinases, and glucose oxi-

dases are selected that are compatible concerning their active pH and temperature range. A combination of two or all three preparation steps with minimal amounts of treatment baths and rinse water showed compatible results in Whiteness, absorbency, dyeability and tensile properties of the treated fabrics.

### Xylanase

In past years, interest in xylanases was concentrated particularly on enzymatic paper bleaching. In textile industry xylanases can destroy the coloured attendant substances of cotton. The quantity of chemicals required in peroxide bleaching could be reduced by this type of enzymatic bleaching.

According to a research "Enzymes for Removal of Non-Cellulosic By-Products of Bast Fibers" the removal of noncellulosic compounds, such as lignin and hemicelluloses, was approached using xylanases in blends with cellulases. For hemp and linen goods very promising results were obtained especially when mechanical action was involved. However, Jute fibers with the highest lignin content of all bast fibers showed a short-lived bleaching effect when these enzymes were used.

At the Hamburg-Harburg (D) University of Technology, a comprehensive screening programmed for isolating extremophile microorganisms (like starch, proteins, and hemicellulose for example) has been implemented which is able to produce enzymes for breaking down biopolymers, alkanes, polyaromatic carbohydrates plus fats and oils. Within the framework of these studies, a range of biotechnologically relevant enzymes like amylases, xylanases, proteases, lipases and DNA polymerases for example have been enriched and characterized.

### Conclusion

The use of various enzymes is in the early stages of development but their innovative applications are increasing and spreading rapidly into all areas of textile processing. Enzyme producing companies constantly improve their products for more flexible application conditions and a more wide-spread use. The textile industry can greatly benefit from the expanded use of these enzymes as non-toxic, environmentally friendly compounds if their effects on the textile substrate and the basic mechanisms involved are better understood.

### References

1. Boyer, P.D. "The enzymes" 3rd ed. Vol. 5. Academic Press, Inc., New York.
2. Fersht, Alan. "Enzyme structure and mechanism". San Francisco: W.H. Brenda The comprehensive enzyme information system. Retrieved 4 April 2007.
3. Briggs G.E., Haldane J.B.S. "A note on the kinetics of enzyme action". *Biochem. J.* 19 (2): PMID 16743508.
4. R.C.Duby. "A text book of Biotechnology". S. Chand & Company press. 2005. ISBN: 81-219-0916-3.
5. Chelikani P, Fita I, Loewen PC. "Diversity of structures and properties among catalases". *Cell. Mol. Life Sci.* 61 (2): 192-208. January 2004.
6. Svendsen A. "Lipase protein engineering". *Biochim Biophys Acta* 1543 (2): PMID 11150608, 2000.
7. Barrett A.J., Rawlings ND, Woessner JF. "The handbook of proteolytic enzymes". Academic Press, 2003. ISBN 0-12-079610-4.
8. M. M. Shrivastava & Rashmi sanghi "Chemistry Of green environment". Narosa publishing house. 2005. ISBN: 81-9319-620-6.
9. Tony Godfrey & Stuart west. "Industrial Enzymology", 2nd ed.
10. Navnath D. Pingale "Eco-friendly textiles through application of bio-technology" <http://textileinformation.blogspot.com>.
11. A Cavaco-Paulo, G Gubit, Graz "Textile processing with enzymes". Woodhead Publishing Limited. August 2003. ISBN-13: 978 1 85573 610 8.
12. Kh. M. Gaffar Hossain, Maria Diaz Gonzalez, Guillem Rocasalbas Lozano and Tzanko, "Multifunctional modification of wool using an enzymatic process in aqueous- organic media". *Journal of biotechnology* Volume 141, Issue 1-2, April 2009. ◆