1 Introduction

Cellulases as enzymes are very important during textile finishing as because of their interesting versatility it is possible to use cellulases on different materials and for different purposes. They work independently but their actions in combination with other enzymes to help them speed up processing are also very important.

The processing of textiles generated by bio-innovative compounds is of current interest, so in this review cellulases were studied and presented as biocarriers during finishing.

Enzymes are biocatalysts, usually proteins of three-dimensional structures. They drive or accelerate chemical reactions in which they remain unchanged.

As with all catalysts, enzymes work by lowering the activation energy for a particular reaction, and thus speed it up by a few million times. The basic function of the enzymes is linking molecules during reactions that operate on complex formations. The enzyme remains unchanged for the entire duration of a reaction which allows it, when one reaction has been completed, to enter into a second unchanged, without affecting the relative energy between the reactants and the products. The specificity of the enzyme as compared to other catalysts is its specificity in terms of stereochemistry and chemical selectivity.

Enzyme activity may be affected by different molecules called inhibitors or activators. Inhibitors are natural or synthetic molecules that reduce or completely destroy the activities of the enzymes. Activators are,
on the other hand, molecules that increase the activities of the enzymes. The enzyme with respect to the structure can be:
- monomeric – contains only a single polypeptide chain, typically one hundred or more amino acids
- oligomeric – comprises a plurality of identical or different polypeptide chains that function as a whole.

Most enzymes are bigger than the molecules that are acting on, and all ten amino acids of the enzyme come into direct contact with the molecule to be transformed. The area where there is direct contact between the enzyme and molecule (substrate) is called the active site. This process is a precautionary measure which ensures enzyme recognition of a specific molecule that serves as a true substrate. There is a theory that for each enzyme there exists a substrate (molecule) that fits it in shape and it thus unlocks or starts. This theory is called lock and key because each key is specific to a particular lock that unlocks (Figure 1) [1].

Figure 1: “Lock and key” model [1]

Another theory explains how the enzyme becomes a blanket that changes its shape to wrap around the substrate and connect to it [2]. Some enzymes are independent units in themselves and thus sufficient to be fully active for achieving activity without additional factors. However, some enzymes need molecules that could help them to accelerate or reduce the activity. These molecules are called co-factors. Co-factors may be inorganic, e.g., metal ions or organic, and as such called co-enzymes [1].

2 Applications of enzymes during textile processes

The first use of enzymes within the textile sector was reported in 1857 when starch-sized cloth was soaked with liquor containing barley. Since the beginning of the 20th century amylase enzymes have been used for de-sizing fabrics.

In the late 1980s, enzymes cellulases were introduced with great success for de-pilling and de-fuzzing cellulose-based fabrics, as well as aging garments made from materials such as denim to obtain the stone-washed look [3]. Enzymes that are used in the textile industry, as well as the effects that they have achieved on the fabrics, are shown in Table 1. The benefits of enzymes in relation to classical agents are multiple, such as: ease of application, mild processing conditions (temperature, pH), safety during operation, biodegradable, does not pollute the environment, and represent an economical option due to shorter processing times compared to other agents. Enzymes are also quite simple and economical to produce which is carried out by fermentation of micro-organisms, and waste products can be used as fertiliser. The benefits of enzymes are contained in the famous environmental, energy and economically compared to traditional processes [4].

Use of industrial enzymes on textiles in the U.S. in 1997 was estimated at 27.1 million dollars, with a tendency for growth. The average annual growth rate was approximately 2% and was expected to reach 32.4 million dollars by 2006 [5].

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3 Cellulases

Cellulase are enzymes widely-applied to cotton, flax, hemp, ramie, viscose, lyocell fibres, in order to improve touch and looks. They are used for the anti-pilling of cotton, defibrillation of lyocell, creating surface effects and super softness [6]. Cellulases break-down cellulose, cotton fibre on the monosaccharide (glucose) and disaccharide (cellobiose) units (Figure 2) [2].

It is present in the digestive juices of certain insects and micro-organisms, and can be found in avocado, peas, Reishi mushrooms [2]. Cellulases have specific strains obtained from fungi (Aspergillus and Trichoderma) or bacteria Trichoderma producing large amounts of endocellulase and exocellulase, whilst Aspergillus produces endocellulase and beta-glucosidase [8]. These enzymes are distinguished from others by their selectivity for cellulose, attacking \( \beta-1.4 \)-glycosidic bonds within the cellulose chain. Hydrolysis of cellulose can result in weight loss and reduction of the breaking force for the cellulose fibres. Achieving certain exact conditions, such as cellulase concentration, \( \text{pH} \), temperature and duration of action of the enzyme, these risks are controlled within the permissible limits. Weight loss rarely exceeds 5%, whilst reducing the breaking force is less than 10% [6].

The cellulase enzyme molecule is composed of up to three types of functionally different domains, as illustrated schematically in Figure 3:
- the catalytically active core, which is large and spherical,
- the linker domain, which is an elongated and flexible spacer and
- a spherical cellulose-binding domain (CBD).

The nature of the core determines the catalytic properties such as endo activity versus exo activity, substrate specificity, and the type of reactive products that are formed. The presence of a CBD is of particular importance for the binding of enzymes onto insoluble and crystalline cellulose and for hydrolytic effects [9]. A model of cellulose with all its specific elements is shown in Figure 4.

Five general types of cellulases based on the type of reaction catalysed [7]:
1. endocellulase randomly cleaves internal bonds at amorphous sites that create new chain ends.
2. exocellulase cleaves two to four units from the ends of the exposed chains produced by endocellulase, resulting in tetra-saccharides or disaccharides such as cellobiose.
   There are two main types of exocellulases [or cellobiohydrolases (CBH)]: CBH I works from the reducing end, and CBH II works from the non-reducing end of cellulose.
3. cellobiase or beta-glucosidase hydrolysates the exocellulase product into individual monosaccharides.
4. oxidative cellulases depolymerise cellulose by radical reactions, as for instance cellobiose dehydrogenase (acceptor).
5. cellulose phosphorylases depolymerise cellulose using phosphates instead of water.
3.1 Cellulases during the scouring of cotton and wool

One of the first steps in the processing of cotton yarn is scouring. Scouring is removing the impurities within raw cotton. Namely, apart from cellulose there are up to 12% various non-cellulosic impurities such as protein, pectin, lignin, fat, wax, natural dyes and others. Scouring of cotton may be carried out by alkaline treatment, extraction with organic solvents or by enzymatic treatment [10].

Enzymatic scouring of cotton fabric can be conducted using different enzymes as an alone or in combination with namely pectinase, cellulase, protease, lipase and others. Cellulase is used especially during the scouring of cotton fabrics. The main function of it is to penetrate the outer layer (cuticle) of the fibre strand and make contact with primary wall. The part of the primary wall at the contact point is hydrolysed and opens up the space for pectinase and other enzymes to react. The result of the synergism is a more effective scouring in terms of speed and evenness. The degree of whiteness of a cotton sample treated with cellulases only is lower by 8–10% than the whiteness of alkaline scoured cotton samples.

Cellulase enzymes are also used in wool scouring. Raw wool consists of natural vegetable impurities which are cellulosic in nature and can be removed by cellulase enzymes treatment. However, the process can partially remove the natural impurities the subsequent chemical treatment may be necessary to complete the pre-treatment of wool fibre [11].

The enzymatic process is environmentally and economically more appropriate because it replaces the use of H$_2$SO$_4$ or HCl in concentration 4–8%. The process is also conducted at much lower temperatures compared to conventional high temperature 100–110°C [12].

The main advantages of enzymatic scouring are environmental premises, biodegradability of wastewaters and no effect on other components within a blend.

Bio-scouring enables energy save, reducing CO$_2$ emissions, saving 20m$^3$ of water per tone of fabric, and 67kg of chemicals on tones of fabrics [13].

3.2 Cellulases in pretreatment of bast fibres

Bast fibres are extracted from the plant stem by a process called “retting” [14]. The impurities are removed through a pre-treatment process and conventional alkali processing applied. Individual enzymes such as pectinase, protease and hemicellulase can be used to remove single component. However, in combination of enzymatic treatment when cellulase enzyme is also added, the pre-treatment process becomes faster and more efficient. In the multi-enzymatic system, the role of cellulase is to remove the surface cellulosic components and to facilitate the other enzymes to react on the specific components which are present in the inner layer of the fibre strands [11]. Different fibres perform differently under the same bio-finishing treatment conditions, e.g. linen, which is highly susceptible to cellulase attack and in some extreme conditions can be destroyed [15].

3.3 Bio-polishing

Most fabrics contain natural cellulose fibres which have small, loose or protruding yarn over their surfaces and this gives them an uneven texture. After frequent wearing and washing the yarn breaks, and their ends are entangled, so peeling occurs, this can make pretty new clothes look old and worn.

Bio-polishing is a process of finishing cellulosic fabrics by being able to remove protruding fibres, thereby improving the texture and appearance of the fabrics, and improve hydrophilic properties, e.g. moisture absorption. This process creates a smooth fabric with resistance to peeling but also improves softness and shine and the appearance of the print area [16]. The process led to a weight reduction of the fibres and a weight loss of 3–5%, but was evaluated as optimum for obtaining a soft handle and a better surface appearance. The first treatment of cellulosic fibres with cellulose was published in 1988 [17]. Bio-polished fabrics look better and last longer. Groups of enzymes called endocellulases are used for bio-polishing. Large molecules of enzyme can not easily penetrate into the interior of fibres but operate primarily on the surfaces of the fibres, which lead to hydrolyses of the cellulose. Enzymatic hydrolysis weakens the fibre ends and subsequent mechanical action removes the loose fibre ends. Effects obtained by this bio-processing are:

- removal of protruding fibres, peels, nodules and lumps,
- achieving a uniform, smooth, clean surface and...
– obtaining of a pleasant shine, smoothness and inner softness.

By removing the nodules and lumps it is achieved a uniform appearance of print with sharp contours and better utilisation of fluorescent whitening agents. The process can be carried out simultaneously in the bath for dyeing because it saves time and energy but it should be done with caution in order to avoid a reduction in depth of colour. Bio-processed cotton materials achieve a clean and smooth surface, softer and more comfortable touch, resistant to washing [16].

3.3.1 Bio-polishing of cotton fabrics

Bio-polishing can be carried out at any stage of wet processing but the more convenient is performed after bleaching. The advantage of this treatment is that it is clean, hydrophilic and easier surface availability for cellulase. Raw fabric would require a higher concentration of enzymes. If bio-polishing would be carried out after dyeing there is the risk of changes in colour shades. Dyes might also reduce the effect of the enzyme and so it would require higher concentrations [18, 19].

The process of bio-polishing requires the following conditions:
- pH range 4.5–5.5,
- temperature of 40–55°C,
- time period 30–60 minutes and
- 1–2% concentration of enzyme per kg of fabric.

Figure 5 shows the differences between cotton fabric before and after treatment with cellulases.

![Figure 5: Bio-finishing of cotton, a. before b. after treatment with cellulases][20]

Enzyme products based on more components were made to hydrolyse cotton cellulose to glucose, therefore containing a number of different cellulases; 4 Endocellulases, 2 exocellulases and 1 cellobiase. Products based on one component were made to achieve a particular effect with only one cellulase, usually endocellulase. The DNA techniques allow manipulation of cellulase genes, and it is now possible to obtain a new targeted cellulase, which can be optimised for specific effects on certain types of fabrics [19]. Endoglucanase achieves a high shrink resistance with less weight loss of cotton material than traditional acid cellulases.

Best results are achieved with low liquor ratio and equipment with relatively high mechanical agitation [21].

3.3.2 Bio-polishing of lyocell

Lyocell fibres compared with other similar cellulosic fibres (viscose, modal, copper) are significantly stronger, especially in wet conditions, have a higher degree of crystallinity, better overall orientation of structure and expressive fibril structures.

Lyocell fibres are special because of their ability to fibrillate, due to fibril structure oriented towards the longitudinal axis of the fibre. This ability to fibrillate the surface layer of fibre is used to achieve special textures and surfaces [22]. Wet processing will cause fibrillation, so it is necessary to clean the surface of the fibrils (Figure 6). Fabric or clothing from lyocell fibres can be bio-polished using a cellulase that cleans the surface of fabric after primary fibrillation but before secondary fibrillation, which provides an interesting look to a fabrics [23]. Lyocell, does not lose much of its strength after treatment [15].

Cellulases for this purpose may act within acidic, neutral or alkaline medium, and each media gives the material specific properties [24].
Increasing the mechanical action at this stage reduces the processing time. The degree of defibrillation can be monitored visually and, if the given effect is unachieved, the procedure is repeated using a small amount of the enzyme usually half of the initial. After enzymatic hydrolysis of clothing produced from lyocell fibres, it has a clean surface without protruding fibres but is unstable during washing. Durable press finishing is required in order to achieve permanent properties during care and maintenance, which is achieved by the process of re-fibrillation.

Re-fibrillation is secondary fibrillation for achieving peach skin effect. This procedure is also possible during the deactivation of the enzyme within an alkaline medium [26]. This was formerly a commercial method for achieving peach skin effect using batch-wise piece-processing over many steps. A brief procedure was developed that involves fibrillation within one bath, dyeing and enzyme treatment, thus shortening the process considerably. While the method was developed for 100% lyocell it is also suitable for dyeing lyocell/polyester blends resulting in further savings compared to a typical blend dyeing profile [27].

Numerous factors influence the bio-polishing of lyocell fibres:
- pH affects the activity of the enzyme (optimal enzyme activity and minimal energy costs),
- the temperature must be optimal,
- the liquor ratio should not be too high because dilution reduces enzyme activity and
- if the mechanical activity is higher, the incubation time with the enzyme will be shorter.

Products with more cellulase components are successful at bio-polishing lyocell fibres. However, in the cases of fabrics from lyocell and ramie or flax blends, one component enzyme product should be applied. It was found that a product with several components of cellulase caused significant damage and reduction in breaking-strengths of these fabrics [23].

4 Processing of jeans, “stone wash”

Today denim products have an important significance in the clothing of all generations and all social groups. One of the common methods of treatment is to achieve a look of washout garments. In order to achieve this artificially, pumice stone (Figure 7) was used for a long time as a single abrasive agent, density lower than 1g/cm$^3$ which floats because of the porosity, and can be found in several places in Europe (Turkey, Greece and Italy). There is also synthetic pumice stone in a variety of sizes and sharpness but are rarely used because it being expensive. The mechanism of action is fairly simple, the stone rubs certain parts of the denim and the constant friction partially removes colour and polished surfaces.

![Figure 7: Pumice stones [34]](image)

The application of bio-technology or the introduction of cellulase enzymes can reduce or completely replace the use of pumice stone, a small amount of enzyme can replace several kilograms of stone [28]. Ideal enzymes for processing denim are endocellulases that have the ability to bind indigo dye and that can have a low capacity of adsorption on cellulose fibres. In this way, re-deposition of indigo dyes to colourless weft is practically negligible. Today, denim is processed with enzymes and their combinations using stones that undermine the structure of the material much less and it becomes soft and a more desirable surface appearance [29].

Using enzymes to achieve the “stonewash” look was first introduced in Europe in 1989. It was accepted and first applied in the United States in 1990. Cellulase activity on the surface layers of cellulose fibres easily removes the indigo dye of denim clothing. Cellulase has many advantages over other agents when finishing of denim:
- reducing the use of stone and thus reducing dust during its operation,
- less machine destruction,
- increases the loading capacity of the machine to 50%,
- reducing the damage to clothes resulted from a stone,
shortening the time required for removing dust from the clothing and,
reducing the problem of wastewater. Very good visual effects are also achieved on denim that can't be obtained by stone abrasion.

There are also five basic factors important for the activity of cellulase, namely; pH, temperature, time, concentration, and mechanics. Acid, neutral and alkali cellulases can be applied for stone washing. Acid cellulases generally provide the best performance within the range of 4 to 5 pH, and such pH being achieved by the addition of acetic acid or another weak acid or a buffer. The action of hot water, cellulase and stone partially remove dye molecules from fabric within the solution which becomes supersaturated. Values of pH ranging from 4 to 5 have impact on the deposits of dye molecules from the supersaturated solution to the weft yarn. After-treatment in a solution of alkaline detergent cannot completely remove back-staining [28]. Back-staining depends more on treatment time than on acid cellulose concentration. More aggressive denim finishing with acid cellulases, requires half the time or five to ten times less enzyme quantity to give the same level of colour removal than with neutral cellulases [30, 31]. Treatment with acid cellulases is carried out at the optimum temperature of 43°C, over a time of 40–60 minutes and the liquor ratio is 1:4–1:8. Whilst working with acid cellulases jeans produces a slightly reddish look, the appearance of jeans during the processing with neutral cellulases is greyish or bluish [32]. In order to avoid back-staining, neutral and alkali cellulases were developed, neutral cellulases have maximum activity at pH6.5.

The alkali stable cellulase from alkalothermophilic Thermomonospora sp. acting in pH8 is also used for the bio-finishing of denim fabric. Cellulase Thermomonospora sp., which is rich in endoglucanase and xylanase, has negligible activity towards crystalline cellulose. Here is a schematic diagramme that indicates the probable mechanism during the enzymatic finishing of denim garments by endoglucanase and xylanase (Figure 8). During the indigo staining of a denim garment the indigo dye particles becomes adhered to the microfibrils present on the surface of the garment (Figure 8b). Endoglucanase acts on the amorphous region of the cellulose forming the protruding hairs on the fabric generally responsible for causing fuzz and pilling on the fabric, and loosens it. The mechanical action in the washing machine in turn removes the loosened fibres to give a final finished product (Figure 8d). As the protruding fibres are removed the indigo particles that also adhere to the surfaces of the protruding fibres are also removed thus giving the fabric a patchy appearance which is preferred (Figure 8d). Xylanases will act on the seed coat fragments and other natural impurities thus giving a final finished touch to the cotton fabric.

Subjective evaluation of the denim fabric after treatment with Thermomonospora sp. rates it to be effective in reducing hairiness, impartation of softness, wash-down effect, and back-staining. It works better than acid cellulase and is comparable to neutral cellulase.

Denim bio-finishing under non-buffering conditions with alkali cellulase is found to show low back-staining, good color contrast, and wash-down effect with better depth of color of the denim fabric on subjective evaluation. Alkaline or neutral conditions are preferable for the enzymatic processing of denim as back-staining takes place under these conditions.

Cellulase Thermomonospora sp. is active and stable under alkali condition and appears to be an excellent alternative for the bio-stoning and bio-finishing of denim garments and therefore compares well with commercial cellulases under alkali conditions.
In addition it causes less back-staining and is effective under non-buffering condition which is more preferable for industrial applications. [33]. During the processing of jeans temperature is an extremely important factor for cellulase activity, so the better operating temperatures range from 48 to 55°C. Increase in temperature leads to the degradation of the enzyme, and thus reducing their activities.

Maximum activity of cellulase is achieved after 90 minutes and afterwards their activity decreases. This is a long process, rare in practice, because the length of time can easily damage the exposed parts of denim clothes. Reduction of processing time requires higher concentrations of the enzyme in order to achieve a good effect but the excessive concentration can have the same bad effect. The more common is the processing time of 20–80 minutes depending on the desired clothes’ appearances.

Optimal concentration of enzyme is 1–4% depending on the desired effect, the higher percentage is not recommended due to the risk of strength reduction.

An important factor affecting the increase in mechanics is the use of stone but to a reduced degree. In practice, the best and special visual effects are achieved by the combined actions of stone and cellulases [34].

Machines for achieving “stone wash” effect on the jeans should have optimal parameters such as speed of rotation for the drum, the ideal being about 30r/min. Higher speed results in non-homogeneous distribution of the enzyme because the textiles are packed in one place on the wall of the drum. The larger diameter of the drum allows for a greater mechanical effect than a smaller diameter. The charge capacity of the machine for good effect is 30–50% of the capacity for the machine. The shapes and sizes of the partitions for drum allow the running of material through the bath in the drum. Compartments must be neither too low nor too high in order not to reduce the effect. Many machines are directly heated by steam which causes very high temperatures in some places that destroy enzymes. In order to prevent this situation, the enzyme should be added only after the desired temperature has been reached. After achieving the desired stone wash effect enzymes should be deactivated. Insufficient deactivation results in further prolonged action of the enzymes on the cellulose, thus reducing the strength and surface mass. Enzyme activity can be blocked in several ways:
- increased pH (pH > 9),
- increased temperature (T > 60°C) for 15 minutes,
- washing of textiles in alkaline detergent solution (pH > 9, T > 60°C) for 15 minutes and
- hypochlorite bleaching to obtain even lighter tones [35].

Hypochlorite bleach or bleaching with hydrogen peroxide can be used for bleaching back staining and to minimise the prolonged action of the enzyme on the textile. The process of bleaching can also change the hue of jeans [36].

5 Cellulases during carbonisation

Fabrics that have polyester-cotton blends of varying proportions are dyed with disperse-reactive or disperse dyestuff and then treated with strong sulphuric acid solution to remove cellulosic components. This process makes the material finer and to change colour. The cellulase enzyme treatment is the best alternative because it hydrolysates the cotton component and removes it from the material. In the cases of cotton rich blends, the traces of the cellulosic part remain on the material and thus require more severe enzymatic treatments. The acidic cellulase enzyme is the best suitable for this application. It is possible to apply appropriate concentrations of the enzyme during the correction of shade obtained by disperses/reactive dyeing blends of cotton with polyester. The same principle is used in the brasso style of printing in which the cellulosic portion is removed from the polyester–cotton blend material after printing [11, 37]. Brasso fabrics are special textiles that are formed by removing parts of cotton after dyeing or printing. This has a dramatic effect on the fabric and the colour impression which can’t be achieved by conventional printing methods. Brasso fabric is often found in Indian saris, but also in fine lingerie [38].

The advantages of carbonisation using cellulase are:
- the process is non-corrosive and non-hazardous,
- less wear and tear to machines,
- eco-friendly process,
- no separate curing is required as in conventional brasso printing and
- no adverse effects on print colour and feel of fabric [11].
6 Conclusions

Cellulases are versatile enzymes that can be used effectively during textile processing to substitute for non eco-friendly chemical treatments. Environmentally, economically and energy they are more acceptable and more suitable for use within the textile industry. Special application of cellulases is used for the world-famous “stone wash” and also for many other lesser-known industrial applications but not less important. Appropriate pH and temperature conditions should be selected for the given fabric and type of cellulase used. Tight control of process conditions that control the hydrolysis of cellulose by cellulase can also enable processors to minimise fabric strength loss. Equipment and process conditions selected should provide the most suitable degree of mechanical action and provide the desired effect.

Cellulases treatment is unique because it multiplies improvements to the properties of textile material within different compositions, thereby extending their field of application regarding blends. Genetic engineering establishes the possibility of cellulase being applicable in acid, neutral and alkaline mediums thus enabling a wide range of activities during various phases of pretreatment and finishing. One of the problems in the application of cellulases is its high price. Possible solutions to this problem are mass application, recovery and reuse, which would compensate for the high initial investments.

The best cellulases perspective compared to other enzymes is reflected in the wide field of application, excellent efficiency and compatibility within multi-enzymatic systems, thereby significantly rationalising processes within the textile industry.

References

Application of Cellulases in the Process of Finishing


