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BIOSCOURING
OF COTTON FABRICS

Ph.D. Thesis

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LIST OF ORIGINAL PUBLICATIONS

This work is based on the following papers, referred to in the text by the Roman numerals given below.


The author in the present thesis had main responsibility for the practical work in all publications.

Publications in Hungarian Journals


Oral and Poster Presentations


### ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>CBH</td>
<td>Cellobiohydrolase</td>
</tr>
<tr>
<td>$\Delta C_{ab}^*$</td>
<td>Chroma difference</td>
</tr>
<tr>
<td>$\Delta E_{ab}^*$</td>
<td>Colour difference</td>
</tr>
<tr>
<td>$\Delta E_{ab}$</td>
<td>Colour evenness</td>
</tr>
<tr>
<td>EDTA</td>
<td>Ethylenediaminetetraacetic acid</td>
</tr>
<tr>
<td>EG</td>
<td>Endoglucanase</td>
</tr>
<tr>
<td>EGU</td>
<td>Endoglucanase Unit</td>
</tr>
<tr>
<td>FPA</td>
<td>Filter Paper Activity</td>
</tr>
<tr>
<td>FPU</td>
<td>Filter Paper Unit</td>
</tr>
<tr>
<td>IU</td>
<td>International Unit</td>
</tr>
<tr>
<td>$\Delta L^*$</td>
<td>Lightness difference</td>
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</table>
1. INTRODUCTION

Preparation and bleaching are among the most energy- and chemical intensive steps of the conventional cotton finishing. About 75% of the organic pollutants arising from textile finishing are derived from preparation of cotton goods. In the conventional preparatory process concentrated sodium hydroxide solution and additional hydrogen peroxide and/or sodium hypochlorite solutions are applied for removing the impurities from raw cotton. Cotton fibres contain approximately 10% by weight of noncellulosic substances, such as waxes, fats, pectins, proteins, noncellulosic polysaccharides, water-soluble inorganics, lignin-containing impurities and colouring materials. Most of them are located in the outer layer of the fibre. By removing the impurities, the preparatory process yields an adequately absorbent and appropriately white material with cellulose content of 99%, but the process generated huge amount of effluent. On the fibre level, oxidative damage may occur and be reflected in a lower degree of polymerisation and decreased tensile strength.

Biopreparation may be a valuable and environmentally friendly alternative to harsh alkaline chemicals for preparing of cotton. In bioscouring the removal of the noncellulosic substances can be achieved mainly by hydrolytic enzymes, such as pectinases, xylanases and cellulases. The bioprocess has several advantages over conventional chemical scouring. Enzymes operate under mild conditions (pH, temperature) with low water consumption and act only on specific substrates.

Numerous studies have been carried out on biopreparation of cotton in the preceding 3–4 years. The results clearly show that in bioscouring of cotton, degradation of pectic substances is one of the most essential processes. Enzymatic degradation of pectin accelerates the removal of waxy materials from the cotton primary wall, thus produces water wettable cotton. However, the degree of whiteness is often less and the process is not suitable for removing seed-coat fragments and mote adequately. By now it is also clear that the greatest obstacle to commercialisation of biopreparation is the removal of matter (stalks, leaves and seed-coat fragments) of vegetable origin.

In spite of the extensive information available and the large number of papers published, numerous questions have remained open concerning the properties of bioscoursed cotton substrates generally and the degradation of pectin and seed-coat fragments particularly. It became clear soon that
if we intend to explain certain phenomena, help process development or contribute to the solution of problems emerging in the practice, we have to concentrate on the basic problem of cotton bioscouring. As a consequence, the main goal of this thesis is to carry out detailed studies on certain aspects of the degradation of pectin and seed-coat fragments.

Desized cotton fabric was treated with different commercial enzymes under a wide range of conditions and the wettability, whiteness, colour evenness and weight loss were studied. Effect of complexing agent on the efficiency of the process was also determined. Attempts were made for determination of the role of complexing agents in the degradation of pectin. In a further step degradation of seed-coat fragment was investigated in details. Fabrics subsequent to biopreparation are generally further subjected to bleaching, dyeing or printing. Therefore, it was particularly essential to characterise the bleachability and dyeing properties of the biopretreated substrates. The results were compared with those obtained in a conventional alkaline scouring.
2. LITERATURE

2.1. Structure of cotton

Cotton is still the most important of the raw materials for the textile industry. Worldwide about 50% of the fibres consumed is cotton (Lewin and Pearce, 1998). Cotton grows as unicellular fibre on seeds (seed hair fibre). The mature cotton fibre forms a highly convoluted flat ribbon, varying in width between 12 and 20 μm. Cotton fibres have a fibrillar structure. Their morphology is illustrated in Figure 1. A thin cuticle that mainly consists of waxes and pectins protects the outside of the fibre. The primary wall in mature fibres is only 0.5–1 μm thick and contains about 50% of cellulose. Noncellulosic constituents consist of pectins, fats and waxes, proteins and natural colorants. The secondary wall, containing about 92–95% cellulose, is built of concentric layers with alternating S- and Z-shaped twists. The layers consist of densely packed elementary fibrils, organised into microfibrils and macrofibrils. They are held together by strong hydrogen bonds. The lumen forms the centre of the fibres (Buschle-Diller, 2003).

Cotton is composed almost entirely of the polysaccharide cellulose. Cellulose is the most abundant renewable polymer today. Chemical composition of cellulose is simple; it is a linear (1→4)-linked polymer of β-D-glucopyranose (Lewin and Pearce, 1998). The degree of polymerisation (DP) of cellulose varies with its source and the processing stage of the cellulosic material. The DP of native cellulose may be as high as 14 000, but the different purification treatments can reduce this to 1000–2000. Three free hydroxyl groups in C2, C3 and C6 per

![Figure 1. Structure of cotton (Nevell, 1995).](image-url)
anhydroglucose unit are available for formation of strong inter- and intramolecular hydrogen bonds. Each cellulose chain has a non-reducing end group at C4 and a reducing end at C1. The latter exhibits the characteristics of both an alcohol and an aldehyde under appropriate conditions (Nevell, 1995).

2.2. Noncellulosic constituents of cotton

Raw cotton fibre, after ginning and mechanical cleaning, is essentially 90–95 % cellulose (typical cotton fibre). The mature cotton fibre has a noncellulosic covering called cuticle that contains waxes, pectins, and proteins left over from biosynthesis. This cuticle is deposited on the primary wall, the first part of the cell wall to be formed (Lewin and Pearce, 1998). The primary wall is about 0.1 μm thick and comprises only about 1 % of the total thickness of cotton fibre. It is illustrated schematically by the cut-out diagram given in Figure 2.

Figure 2. Cotton fibre primary wall matrix, illustrating the concentration gradient of noncellulosic material

The major portion of the noncellulosic constituents of cotton fibre is present in or near the primary wall. The exact percentage of each component of the primary wall is determined by the type of the cotton plant, its origin, the growth conditions, and the degree of maturity (Lewin and Pearce, 1998). The composition of a mature cotton fibre is given in Table 1.
Table 1. Composition of the components identified in cotton fibres and in the cuticle (Lewin and Pearce, 1998; Hardin et al., 2004).

<table>
<thead>
<tr>
<th>Components</th>
<th>Composition of the whole fibre [% of the dry weight]</th>
<th>Cuticle [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cellulose</td>
<td>88 – 96</td>
<td></td>
</tr>
<tr>
<td>Protein</td>
<td>1.1 – 1.9</td>
<td>30.4</td>
</tr>
<tr>
<td>Pectin</td>
<td>0.7 – 1.2</td>
<td>19.6</td>
</tr>
<tr>
<td>Wax</td>
<td>0.4 – 1.0</td>
<td>17.4</td>
</tr>
<tr>
<td>Ash</td>
<td>0.7 – 1.6</td>
<td>6.5</td>
</tr>
<tr>
<td>Others (e.g. seed-coat fragment)</td>
<td>0.5 – 1.0</td>
<td></td>
</tr>
</tbody>
</table>

Of all the noncellulosic constituents, the nitrogen-containing compounds constitute the largest percentage when expresses as percent protein. The nitrogenous material occurs principally in the lumen of the fibre, most likely being protoplasmic residue; a small portion is also extracted from the primary wall. Cotton fibre and its primary wall both contain proteins/peptides, free amino acids, and most likely nonprotein nitrogen. The free amino acids that have been detected are glutamic acid, aspartic acid, valine, serine, and threonine (Lewin and Pearce, 1998). It is suggested that the brown colour of cotton and dark brown colour of kier liquors are associated with proteins (Ward, 1955).

Pectin is located mostly in the primary wall of the fibre. It is composed of a high proportion of D-galacturonic acid residues, joined together by $\alpha(1\rightarrow4)$ linkages. The carboxylic acid groups of some of the galacturonic acid residues are partly esterified with methanol (Dey and Harborne, 1997). Pectic molecule can be called a block-copolymer with alternating the esterified and the non-esterified blocks (Jarvis et al., 1984). In the primary cell wall pectin is covalently linked to cellulose or in other plants to hemicellulose, or that is strongly hydrogen-bonded to other components. On the other hand, pectin undergoes self-interaction to some extent, it can forms crossbridges by the help of calcium ions (Dey and Harborne, 1997). The crosslinks are formed between the non-esterified or little methyl-esterified galacturonan blocks with negative charge and calcium ion with positive charge by electrostatic interactions (“egg-box” model) (Demarty et al., 1984; Dey and Harborne, 1997).
All the *waxes* take place on the surfaces of the cotton fibre, in the primary wall, which is responsible for the hydrophobic nature of unscoured cotton. The waxes consist of various hydrocarbons, fatty alcohols, fatty acids and their esters. The presence of waxes is detrimental in the chemical processing of cotton yarns and fabrics because it interferes with wetting of the fibre and penetration of reagents (bleaching agent, dyes, etc.). After scouring the wax content of the fibre is about 0.15%. Because of its inhomogeneous distribution, the fibre has a hydrophilic character (Lewin and Pearce, 1998; Trotman, 1968).

The inorganic salts (phosphates, carbonates and oxides) and salts of organic acids present in raw fibre are reported as percent *ash*. The ash itself is highly alkaline. The ash contents of cottons are highly variable in composition and quantity, arising in differences in soil and agricultural practices. During the production of cotton, the plant absorbs potassium and other metals as normal nutrients. The following metals are found in raw cotton fibre: potassium, calcium, magnesium, sodium, iron, manganese, copper, zinc. Metals in cotton are of importance of processors, because they can contribute to problems in bleaching and dyeing. Decomposition of peroxide can be catalysed by iron and copper ions, which may induce fibre damage. Insoluble calcium and magnesium salts can interfere with dyeing (Lewin and Pearce, 1998).

### Table 2. Chemical composition of cotton seed-coat fragments (Csiszár et al., 1987).

<table>
<thead>
<tr>
<th>Components</th>
<th>Amount [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wax</td>
<td>5.6</td>
</tr>
<tr>
<td>Lignin</td>
<td>28.3</td>
</tr>
<tr>
<td>Holocellulose, including</td>
<td>41.3</td>
</tr>
<tr>
<td>α-cellulose</td>
<td>23.0</td>
</tr>
<tr>
<td>pentosans</td>
<td>7.2</td>
</tr>
<tr>
<td>Ash</td>
<td>12.8</td>
</tr>
<tr>
<td>Moisture</td>
<td>8.3</td>
</tr>
<tr>
<td>Others</td>
<td>3.7</td>
</tr>
</tbody>
</table>

2. Literature
Seed-coat fragments (Figure 3) present on the surface of cotton fabric are the most resistant impurities of cotton. Seed-coat fragments are part of the outer layer of cotton seed and they are formed from mature or immature seeds during mechanical processing. They are usually black or dark brown and may or may not have fibres and linters attached (Annual Book of ASTM Standards, 1980; Verschraege, 1989). Chemically seed-coat fragments consist of two main components: lignin and holocellulose (Table 2). The inorganic content of seed-coat fragments is significant, there are potassium, calcium, and magnesium compounds in large scale (Csiszár et al., 1987).

Similarly to other plants, hemicelluloses are also present in seed-coat fragment. They are polymeric molecules that contain components other than glucose, such as xylose or galactose residues. Shorter oligomeric molecules are also present. These molecules coexist readily with cellulose but have different properties, especially solubility (Lewin and Pearce, 1998).

Seed coat fragments are more troublesome in pretreatment than the other non-cellulosic constituents of the cotton fibres. Although, the different mechanical cleaning steps remove majority of the seed-coat fragments, a certain amount of these impurities may adhere to the fibres and may be present even in the final fabric. In the traditional chemical processes their removal requires more concentrated chemical solutions and longer steaming periods.
2.3. Enzymes and their use in processing of cellulosics

Enzymes are high molecular weight proteins that are produced by living organisms. They are capable of catalysing chemical reactions in biological processes and hence are known as ‘biocatalysts’. Generally enzymes contain an ‘active site’, where the reaction is carried out, and a ‘binding site’, which links to the substrate. In order to catalyse a reaction, the enzyme molecule has to form a complex with the substrate. The enzyme has a quite specific three-dimensional shape. This shape and other factors, such as the location of the active site on the enzyme, control the substrate specificity of the molecule.

The enzyme is connected to the substrate by ‘lock and key mechanism’ (Figure 4). The bioreaction takes place in the enzyme-substrate complex reducing the activation energy, and as a consequence, the reaction speed is multiplied. Finally, the complex disintegrates releasing the reaction products, and the original enzyme is available again. Generally enzymes work in mild reaction conditions: atmospheric pressure, low temperature and slight acidic, neutral or alkaline pH. They are very sensitive molecules, therefore the assurance of the optimal reaction conditions is needed during the whole process (Shukla et al., 2000; Etters and Annis, 1998).

The efficiency of the enzyme-catalysed reactions depends on a number of factors. Enzymes can work with maximum activity only in a specific narrow range of temperature. Increasing the temperature, the activity in...
creases due to increased possibilities of the enzymes coming closer to substrate molecules. On the other hand, denaturation of the enzyme protein due to heat is also accelerated. Most enzymatic activities are extremely sensitive to $pH$. Any increase or decrease of $pH$ results in lowered reaction rates. A variation of $pH$ during the reaction may bring about an alteration of the protein structure with a denaturing effect or the ionisation of the active site. In order to avoid deviations of the $pH$ during the reaction it is suggested to buffer the medium. Certain enzymes require some specific bivalent metallic cations as activators that stabilise the structure of the enzyme-substrate complex. Certain chemicals such as alkalis, acid liberating agents, heavy metal cations, oxidising or reducing agents, however, acts as inhibitors of the enzymes blocking or destroying the useful groups of the molecules (Shukla et al., 2000).

Textile industry uses various chemical agents in the different wet processes. These chemicals, after their use, cause pollution in the effluents; some of them are corrosive that could damage equipment and the substrate itself. However, by introducing enzymatic processes an environment friendly production can be ensured.

The serious wastewater pollution caused by conventional textile finishing has oriented the research towards application of enzymes in textile wet processes. The enzymes are non-toxic and environmentally sound. Being natural products, they are completely biodegradable and accomplish their work quietly and efficiently without leaving any pollutant behind. One of the oldest technology being used today is based on amylase-catalysed hydrolysis of the starch size. And in the last two decades several other enzymatic processes have also been developed for the different wet processing of textile goods in wide-ranging operations from cleaning preparations to finishing processes. Cellulases, hemicellulases and pectinases (hydrolys) acting on native cellulosic fibres (cotton, flax, hemp, jute, etc.) became the target enzymes in textile bioprocessing.

The production of “aged” denim garments with cellulases is the most successful enzyme process that has emerged in the textile industry in the last decades. Each year approx. 1 billion jeans are produced and a big part of it is further finished to stonewashed fashion. Cellulase enzyme removes the cotton fragments from the surface of the garment and facilitates the abrasion of indigo dye from the fibre surface. Traditional stonewashing process carried out by pumice stones was vastly replaced by enzymatic biostoning and fading or combined procedures (Lange et al., 1993).
Biofinishing or biopolishing represents another application of cellulases for non-denim cellulosic fabrics and garments. To various extents, all cellulosic materials have a tendency to fuzz formation. Fuzz is the designation for short fibres protruding from the surface of the yarn and fabrics. Traditionally, chemical agents (cationic surface-active compounds) are used to decrease the fuzz formation. In the environmentally-friendly process cellulosic fabrics are subjected to a treatment with cellulolytic enzymes, and the action of cellulases removes the small loose fibre ends that protrude from the fabric surface as well as improves handle properties of the fabrics (Cavaco-Paulo et al., 1998).

Xylanase enzymes have not been applied in the textile procedures before, but they are well-known in the pulp and paper industry. Xylanase treatment of the pulp means an indirect bleaching method, rendering the fibres more accessible to bleaching chemicals and leading to a more efficient delignification. Lignin-carbohydrate linkages have been proposed to restrict the chemical removal of lignin from the pulp. Xylanase enzymes attack xylan, chemically bonded to lignin or degrade reprecipitated xylan, deposited on the fibre surface during cooking, resulting in an improved removal of coloured lignin substances. Xylanase enzyme-aided bleaching is one of the most successful and widely used environmentally friendly biotechnological processes (Viikari et al., 1996).

Recent results indicate that certain enzymes may be used effectively in the cleaning procedure of cotton. The scientific interest in this process is reflected in the number of papers published during recent years describing biopreparation results obtained, using various enzymes from different sources. But enzymatic biopreparation of cotton represents a fairly new approach and is still mostly in the development stage.

In the following part the characteristic features of pectinases, cellulases and xylanases, as well as the mode of their action on cellulosics are discussed in details, since these enzymes are used in the experiments.

2.4. Mode of action of pectinases, cellulases and xylanases

Pectinases catalyse the degradation of pectin. The total degradation is resulted by the harmonised work of several enzymes with different activities. These enzymes are in synergism with each other. There is a nondepolymerase in pectin degrading enzyme system: pectin-esterase. This enzyme catalyses the cleavage of ester bond of poligalacturonan, as a
consequence the degree of esterification decreases. Free carboxyl group and methyl alcohol are produced in the reaction (Bailey and Pessa, 1990; Henriksson et al., 1999). *Polygalacturonase*, which is a depolymerase enzyme, catalyses the cleavage of α(1→4) bonds in pectic polymer chain, releasing water and reducing groups at the chain ends. Exopolygalacturonase works at the end of the chain, while endopolygalacturonase works randomly within the chain (Bailey and Pessa, 1990; Schejter and Marcus, 1988). *Transeliminase* or shortly *lyase* (depolymerase) catalyses the cleavage of α(1→4) bonds in polygalacturonan chain without releasing water and creating a double bond between the C4 and C5 atoms. Endo and exo enzymes work within or at the end of the chain, respectively, similar to the polygalacturonases (Sakamoto et al., 1994).

Cellulases catalyse the degradation of cellulose. All cellulases have an identical chemical specificity towards the β(1→4) glycosidic bonds, but they differ in terms of the site of attack on the solid substrates. This difference in their mode of action has been defined as *exoglucanase* and *endo-glucanase* activities. The exoglucanases, or cellobiohydrodrolases (CBH) cleave cellobiose units from the end of the polysaccharide chains and typically exhibit relatively high activities on crystalline cellulose. *Trichoderma reesei* produces two different cellobiohydrodrolases. CBH I is presumed to attack the cellulose chains from their reducing ends and CBH II from their non-reducing ends. The mechanism of action of cellobiohydrodrolases has been investigated in detailed. These enzymes catalyse the hydrolysis of the glycosidic bonds by general acid catalysis. The reaction involves two essential carboxylates, the proton donor and the nucleophile. Depending on the spatial arrangement of these two residues, the reaction proceeds either by retention or by inversion of anomeric configuration at the C1 carbon. *T. reesei* CBH I has been shown to be a retaining enzyme, whereas CBH II is an inverting enzyme (Koivula et al., 1998).

The endoglucanases (EG) make more random cuts in the middle of the long chains, thereby producing new chain ends for the cellobiohydrodrolases to act upon. The endoglucanases seem to prefer the amorphous regions of cellulose and in contrast to the cellobiohydrodrolases, can also hydrolyse substituted celluloses, such as carboxymethylcellulose and hydroxyethyl-cellulose. Finally, β-*glucosidases* cleave cellobiose and other soluble oligosaccharides to glucose, which is an important step since cellobiose is an end-product inhibitor of many cellulases (Koivula et al., 1998).
Xylanases catalyse the hydrolysis of xylan, the major constituent of hemicellulose. Xylans are heteropolysaccharides with a homopolymeric backbone chain of \((1\rightarrow4)\)-linked \(\beta\)-D-xylopyranose units. The backbone may consist of O-acetyl, \(\alpha\)-L-arabinofuranosyl, \(\alpha(1\rightarrow2)\)-linked glucuronic or 4-O-methylglucuronic acid substituents. Two types of xylanases are distinguished, one is a non-branching, which does not liberate arabinose, while the other is a debranching, which liberates arabinose from the side chain substituents in addition to cleaving main chain linkages. *Endo- and exoxylanases* work within or at the end of the chain, respectively. The hydrolysis reaction of xylan catalysed by xylanases, proceeds through an acid-base mechanism with the retention of anomeric configuration in the product. Xylans are present in a partly acetylated form in various plants. The O-acetyl groups present at C2 and C3 positions of xylosyl residues, inhibit xylanases in completely degradation, probably by steric hindrance. The synergetic action of *acetylxylan esterases* and xylanases is therefore essential for the complete hydrolysis of acetylxylans (Kulkarni et al., 1999).

### 2.5. Biopreparation of cotton

Raw cotton contains about 90% of cellulose and various noncellulosics such as waxes, pectins, proteins, fats, lignin-containing impurities and colouring matter. The goal of the cotton preparatory process is to remove the hydrophobic and noncellulosic components and produce highly absorbent fibres that can be dyed and finished uniformly. In the conventional energy and chemical intensive process concentrated sodium hydroxide solution and additional hydrogen peroxide and/or sodium hypochlorite solutions are applied for removing the impurities from greige cotton. The mild reaction conditions offered by enzymatic treatment provide an environmentally friendly alternative. Pectinases, cellulases, proteases and lipases have been investigated most commonly and compared to alkaline scouring.

#### 2.5.1. Bioscouring with pectinases

Some of the researchers have reported that pectinase treatment alone results in adequate wettability (Traore and Buschle-Diller, 2000; Buchert and Pere, 2000; Li and Hardin, 1998b; Hartzell and Hsieh, 1998b), however, others have found only a little improvement in water absorbency (Hartzell and Hsieh, 1998a). Tzanov and his co-workers investigated two types of pectinases: an acidic and an alkaline pectinase. Both pectinases
are found to be equally efficient in terms of improved absorbency as the conventional alkaline scouring. The alkaline pectinase has similar performance to the acidic pectinase, however the former works on lower concentration, which is beneficial from the economic point of view (Tzanov et al., 2001). Yachmenev and his co-workers obtained better absorbency and whiteness after the treatment with alkaline pectinase than with acidic pectinase (Yachmenev et al., 2001).

The pectin content of cotton fibre can be decreased by about 30% during the pectinase treatment. Removal of pectin results in lower amounts of waxes on the cotton surface and subsequently in improved water absorbency of the fabric, which supports the hypothesis of chemical linkage between pectin and waxes. The enzymatic treatment has no effect on the tensile strength (Buchert and Pere, 2000), moreover it enhances slightly the whiteness (Traore and Buschle-Diller, 2000).

Pectin acts as a sort of cement or matrix that stabilises the primary cell wall of the cotton fibres. During incubation the enzymes will degrade pectin, thereby destabilising the structure in the outer layers. The weakened outer layers can be removed in a subsequent wash process (Nierstrasz and Warmoeskerken, 2003).

Pectinase treatment modifies the morphology of cotton fibres. Raw cotton has smooth appearance with characteristic parallel ridges and grooves. The smooth surface without irregularities is a reflection of the amorphous stage of the cuticle. After pectinase treatment the fibre surface becomes perforated at first, and further treatment results in cellulose fibrils protruding from the surface of the fibre. The cause of the changes can be proposed as pectinase digesting the pectins, which are part of the connecting structure holding the cuticle on the fibre surface (Li and Hardin, 1998a).

Pectinase treatment subsequent to a hot water pretreatment can further improve the wettability of the fabric. Waxes have a melting point about 70°C, therefore during pretreatment they melt and disperse into the treatment bath or they are redistributed on the fibre surface. The thickness of the fabrics increases, moreover lightness and weight loss are slightly increase by the water pretreatment. Subsequent pectinase treatment results in further changes neither in thickness, nor lightness. Yarn strength is not altered by the enzyme treatment (Hartzell and Hsieh, 1998a, 1998b).
Sorption and dyeing properties of the pectinase scoured cotton fabrics have also been investigated and compared with those of the fabrics pretreated with alkaline solution (Etters et al., 1999a, 1999b). Results prove that there is no difference in water sorption between the bioscoured and conventionally scoured fabrics. However, the wicking rate of the bioscoured fabric is greater than that of the alkaline scoured fabric. Dying with direct dyes does not result significant difference in colour depth (K/S) and colour yield ((K/S)/c) between alkaline scoured and bioscoured samples. Uniformity of the bioscoured and dyed samples is better than that of the conventionally scoured and dyed substrates.

Novozymes, Bayer and Dexter Chemical Corporation have introduced an enzymatic alternative for scouring woven and knitted cotton fabrics in the textile industry on the basis of an alkaline pectinase (Bioprep 3000) produces by a genetically modified *Bacillus* strain. The bioscouring process results in textiles being softer than those scoured in the conventional sodium hydroxide process, however the degree of whiteness is often less and the process is not suitable for removing seed-coat fragments and mote adequately (Nierstrasz and Warmoeskerken, 2003).

2.5.2. Bioscouring with cellulases

Cellulase enzyme treatment improves significantly the water wettability and water retention capacity of cotton fabrics (Li and Hardin, 1998b; Hartzell and Hsieh, 1998a). Comparing cellulase to the noncellulosic enzymes, it is obvious that cellulose causes greater weight loss and decreases the yarn tenacity. Pretreatment with hot water enhances the effectiveness of cellulase, similar to pectinase. Morphology of the fibres changes significantly after the treatment. Progressive hydrolysis of cellulose after cuticle removal results in protruding fibrils and cavities on the surfaces of the fibres. Cracks are more frequently observed in cellulase treated fibre than in noncellulosic enzyme treated fibre (Traore and Buschle-Diller, 2000). Cellulases peel off the cuticle by digesting primary wall cellulose, then they have the opportunity to act on the internal cellulose layers and digest them gradually (Li and Hardin, 1998a).

2.5.3. Bioscouring with proteases

Proteases are enzymes that catalyse the hydrolysis of proteins. Different kinds of proteases have been investigated in cotton bioscouring (Hartzell and Hsieh, 1998a; Buchert and Pere, 2000). Results prove that proteases have only a slight effect on improving wettability, however 50% of the pro-
tein can be removed from the fibres. Protease appears to be effective in removing the proteinaceous materials only from the lumen but not from the surface. Proteins of the surface layers are imbedded beneath the pectin materials, therefore they are inaccessible for the enzymes. However, Lin and his co-worker have shown that there are some proteases, which improve fabric wettability to the same level as alkaline scouring. These proteases are capable of accessing the surface proteins without any pretreatment (e.g. hot water) to alter the surface waxes to dislodge the hydrophobic surface compounds (Lin and Hsieh, 2001).

Protease treatment causes no change in the tensile strength and surface friction (Buchert and Pere, 2000; Lin and Hsieh, 2001). Moreover, some proteases improve the lightness of the fabric supporting the role of protein in the coloration of cotton (Buchert and Pere, 2000). Others produce slight yellowness (Lin and Hsieh, 2001).

2.5.4. Bioscouring with lipases

Lipases catalyse the hydrolysis of fats. Common fats are esters of glycerol and fatty acids. Lipases attack the ester bonds regenerating water-soluble glycerol and water-insoluble fatty acid. Lipase treatment does not improve water wetting and retention properties of cotton. It may be explained by either the low accessibility of waxes in the fibres, or the less susceptibility of waxes to enzymatic action (Hartzell and Hsieh, 1998a; Buchert et al., 1998). Lipase treatment results in reduced friction coefficient. It does not affect the yarn strength (Buchert et al., 1998).

2.5.5. Combined enzyme systems in bioscouring of cotton

Using different kind of enzymes in the same treatment bath synergetic effect can be observed in the bioscouring process. Pectinase and cellulase enzymes are applied together most frequently. The combined treatment improves the wetting properties of the cotton fabrics significantly and requires lower amounts of enzymes (Li and Hardin, 1998b). Cellulase apparently assists the action of pectinase by increasing the accessibility to the pectin materials. Removing the pectins should release other noncellulosic components residing on the fibre surfaces (Hartzell and Hsieh, 1998a). In other words by cleaving the biological glue (pectates) in the primary cell wall, pectinase makes the noncellulosics more easily removable (Durden et al., 2001).
Pectinase in combination with cellulase or lipase or xylanase was also investigated. After each combined treatment better absorbency is obtained than the pectinase treatment alone. The best wettability is measured when all the enzymes are applied in the same treatment bath. Analysis of the pectin with Ruthenium Red does not indicate significant differences in pectin removal. All samples show small amounts of pectins and proteins unevenly distributed on the fibre surface. The whiteness of the fabrics is slightly enhanced, and the dyeing with direct dyes yields uniform results and comparable colour strength. Each sample shows significantly softer hand with basically unchanged tensile properties, except for the treatments where cellulase is also present in the treatment bath (Traore and Buschle-Diller, 2000).

2.5.6. Effect of reaction parameters on the efficiency of bioscouring

Many reports confirm that the surfactant has a major role during enzymatic process (Li and Hardin, 1998b; Lenting et al., 2002; Sawada et al., 1998, 2001). Ionic surfactants are particularly effective enzyme denaturants. Anionic surfactants may form complexes with proteins and disrupt their structure. Cationic surfactants may also form complexes with proteins, but reduced affinity. However, nonionic surfactants are compatible with enzymes, they do not reduce the catalytic activity, unless their concentrations in the solution greatly exceed their critical micelle concentration. The surfactant reduces the surface tension of cotton assisting enzymes to penetrate micropores or cracks and helping them to orient themselves in favourable positions for the catalytic functions. After the reaction the enzymes re-enter the solution to bind to new sites again with assistance of the nonionic surfactants.

Lenting and his co-workers have reported recently that after the pectinase treatment of cotton fabrics in the presence of a surfactant, the measured wetting time is the result of two contrary phenomena: the absorption of the surfactant on the hydrophobic cuticle and the removal of the cuticle substances upon pectin hydrolysis. Moreover the impact of rinsing temperature after enzymatic treatment is more than that of the surfactant. Increasing the rinsing temperature the hydrophobic material removal overrules the impact of surfactant absorption and the absorbency improves. This result is related to the melting point of the different wax compounds present in the cuticle. A combination of a high surfactant concentration in the enzymatic incubation and high temperatures in the rinsing process afterward could result in adequate absorbency. The most crucial factor in the whole bioscouring process is the efficient removal of
the enzyme-weakened cuticle compounds in the rinsing phase. In combination with an optimal rinsing process, a processing time of several minutes of enzymatic degradation of pectin will be sufficient (Lenting et al., 2002).

Effect of mechanical *agitation* on the effectiveness of the enzyme treatment has also been investigated in detail (Hartzell and Durrant, 1998c; Li and Hardin, 1998a; Traore and Buschle-Diller, 2000; Cavaco-Paulo et al., 1996). Generally it is found that agitation may increase the enzyme activity and the efficiency of scouring. However, using mechanical agitation with high shear forces, the enzyme can be deactivated, presumably by disrupting the characteristic three-dimensional structure that makes the enzyme substrate specific. The positive effect of agitation may be attributed to the changes in boundary layer. Sorption or desorption of diffusants on the fibre can be influenced by the rate of flow of the processing bath past the fibre surface. The reason for this effect is found in the existence of a flow-related hydrodynamic boundary layer. As agitation increases boundary layer effects become small and diffusion of the decomposition products from the reaction sites is not impeded by boundary layers, resulting in faster and more efficient catalytic action (Li and Hardin, 1998a).

**Hartzell** and her co-worker have generated fabric-to-fabric agitation treating two samples in a single reaction container with pectinase enzyme. The water absorbency values were equal to those obtained from single treated fabrics, despite a 50% reduction in enzyme concentration. Furthermore fabric-to-fabric agitation does not cause any additional loss in strength, tenacity or elongation (Hartzell and Durrant, 1998c).

Another possibility for improving the enzyme transport to the substrate surface beside mechanical agitation is the sonication. Introducing ultrasonic energy to the treatment bath results in very small bubbles in the liquid, which generate powerful shock waves when they collapse. It causes powerful agitation in the liquid border layer and as a consequence, it improves the transport of the bulky enzyme molecules towards the fibre surface and increases the overall reaction rate. At laboratory scale, introducing ultrasonic energy into the reaction chamber during enzymatic scouring of raw cotton fabric significantly improves the pectinase efficiency. Adequate water absorbency was observed without loss of tensile strength of the fabrics (Yachmenev et al., 2001).
2.6. Aims of the study

The aim of this work was to investigate the enzymatic degradation of the impurities in greige cotton fabric in order to achieve an adequately absorbent and appropriately white substrate with minimum impacts such as strength and weight losses.

The specific topics of the study were:

- To investigate the effect of different commercial enzymes, i.e. cellulase, hemicellulase-pectinase and xylanase enzymes on biopreparation of cotton fabrics.

- To study the synergetic effect between enzyme and chelator on degradation of the pectin and lignin-containing impurities (seed-coat fragments) of cotton.

- To investigate the bleachability and dyeing properties of the biopretreated cotton fabric.
3. MATERIALS AND METHODS

3.1. Substrates and enzymes

*Greige cotton print cloth fabric* obtained from Testfabric Inc., NJ. was used for the tests after amylase enzymatic desizing. *Spinning blowroom waste* (a combined waste produced during the mechanical steps of cotton purification) was selected to model the impurities. It consists of stalks, leaves and seed-coat fragments of the cotton. Before enzymatic treatment, the substrate was washed in distilled water to extract the water-soluble constituents and air dried. 100 per cent *greige linen fabric*, plain-weave, supplied by Pannon-Flax Linen Weaving Co. Győr, Hungary, was also tested as an additional substrate for studying the effect of EDTA on the pectin degradation in bioscouring.

The applied *enzymes* were an acidic cellulase (*Celluclast 1.5 L, Trichoderma reesei* origin), an acidic pectinase (*Viscozyme 120 L, Aspergillus sp.* origin) and a pure xylanase (*Pulpzyme HC, Bacillus sp.* origin). They were gifts from Novozymes, Copenhagen, Denmark. Enzyme activities are given in chapter 4.3.1. in Table 3.

3.2. Treatments

*Enzymatic treatments* were carried out in non-agitated and agitated systems, respectively, at pH 5.0 for the enzymes *Celluclast 1.5L* and *Viscozyme 120L*, or at pH 7.0 for the enzyme *Pulpzyme HC*; enzyme concentration of 1–4 g/l; treatment times of 1 h; incubation temperature of 50 °C; and a nonionic surfactant concentration of 1 g/l. In the agitated system the enzymatic treatment was accomplished in a Scourotester laboratory unit. When the effect of the chelating agent was tested, ethylenediaminetetraacetic acid (EDTA) was added to the enzyme solution (I). Investigating the role of EDTA, different kinds of treatments have been applied: EDTA-only, enzyme-only, EDTA pretreatment and subsequent enzymatic treatment, without drying between the two steps and enzyme and EDTA applied together in one bath (III, IV). After enzymatic treatment the substrates were washed in hot distilled water to deactivate the enzyme and then air-dried. Reference treatments were carried out similarly without enzyme addition.
Caustic scouring was performed in a Mathis Labomat Typ BFA12 equipment with 50 g/l sodium hydroxide solution. Hydrogen peroxide bleaching was carried out in the same equipment, the bleaching solution contained 2 ml/l of 30 % hydrogen peroxide (II).

Dyeing was carried out in the Mathis Labomat by bath exhaustion procedure, with a liquor ratio of 1:20. A heterobifunctional reactive dye (Dye A, Sumitomo Co. Japan) was used at four dye concentrations (0.2, 0.5, 1.0 and 2.0 %) based on the weight of the cotton fabric. After dyeing the samples were rinsed in distilled water and air dried (II).

3.3. Analysis of enzymes and substrates

The enzyme activities were measured by internationally recognised methods (Bailey and Nevalainen, 1981; Bailey et al. 1992; Ghose et al., 1987; Kubicek et al., 1982; Miller et al., 1959; Schejter and Marcus, 1988). The influence of EDTA on the activity of the enzymes was tested by performing the standard activity assay in the presence of EDTA at the EDTA-enzyme ratio of 1; 3; 5; 6; 8.6 mM EDTA/ml enzyme.

Weight loss of the substrates was determined by weighing the samples (i.e. fabrics, seed-coat fragments) before and after the treatment following 24 h of conditioning at 20 °C and 65% rh. Release of reducing sugars during the enzymatic treatments was determined in the reaction liquor using the 3,5-dinitrosalicylic acid reagent (Miller et al., 1959). Fabric wettability was determined by water dropping test, counting the elapsed seconds between the contact of the water drop with the fabric and the disappearance of the drop into the fabric. Colour measurement was performed by using a Hunterlab Color QUEST (D65/10°) colorimeter. The colour evaluation was done according to the CIELab colour space. The substrates were characterised by L* a* b* colour coordinates. The efficiency of the processes (e. g.: pretreatment, bleaching), as well as the divergence between the reference and enzymatic treated samples were characterised by the colour difference (ΔE_{ab*}) values. Colour evenness of the treated fabrics and fastness to washing of the dyed samples were also investigated (II). The metal ion content of the substrates and the enzymes was determined by inductively coupled plasma optical emission method (ICP-OES) (I, III, IV).
4. RESULTS AND DISCUSSION

4.1. Bioscouring of cotton fabric

Greige cotton contains various noncellulosics such as waxes, pectins, proteins, organic acids, lignin-containing impurities and colouring matter. The goal of the cotton preparation process is to remove these hydrophobic noncellulosic components and produce highly absorbent fibres with appropriate whiteness. Wettability, absorbency, whiteness and uniformity of the pretreated fabrics have significant effect on the efficiency of the subsequent processes, therefore these parameters have been investigated after bioscouring in details.

4.1.1. Wettability (II)

Greige cotton fabric is hydrophobic and it can be characterized by a wetting time of more than 60 seconds. Desized cotton fabric is also hydrophobic, but it has a measurable wetting time near 10 seconds owing to the desizing process with amylase and surfactant. All of the enzyme treated fabrics are hydrophilic and each has a wetting time less than one second. It can be stated that the enzymatic treatments in the presence of an appropriate surfactant improve water wettability of the cotton fabric to a level, which is adequate to dyeing, printing and finishing, as well as result in homogeneously absorbent fabric. The water absorbency achieved with enzyme is sufficient and equal to that obtained by conventional scouring process.

4.1.2. Colour differences, colour evenness and bleachability (II)

Removal of natural colouring matters from the cotton fibres was evaluated by colour measurement of the fabric. Conventional scouring results in extremely high colour difference (8.4) with desized fabric. An analysis of the constituent of $\Delta E_{ab}^*$ reveals that alkaline treatment increases the lightness and decreases the colour content significantly (Figure 5). Colour difference values of the enzyme treated samples with desized fabric, however, are significantly lower (0.4–1.1), indicating less effective removal of natural colouring matters from cotton fibres.

Comparing the colour of enzyme treated fabrics with that of the alkaline scoured fabric, we see dramatic colour differences (Figure 6). Conventionally scoured fabric is significantly lighter and less coloured than all of the pretreated samples.
4. Results and Discussion

Figure 5. Lightness and chroma differences of the conventionally scoured and biopretreated samples with desized fabric.

Figure 6. Colour difference between biopretreated and conventionally scoured fabrics after bioscouring and subsequent hydrogen peroxide bleaching.
Treatments with enzymes do not influence the colour evenness of the fabrics and do not cause inhomogenities in the substrates. Most of the calculated $\Delta E_{ab}^*$ values are 0.2, thus, human eye can not make differences in colour of the pretreated fabric samples. It means that there are not visually noticeable differences in colour of the pretreated samples.

The effect of hydrogen peroxide bleaching applied after the different pretreatments (enzymatic, caustic scouring) is examined on the fabric properties. Comparing the colour differences between enzyme treated and conventionally scoured fabrics, measured after pretreatment as well as after hydrogen peroxide bleaching (Figure 6), it becomes obvious that hydrogen peroxide bleaching significantly reduced the colour differences between the alkaline and biopretreated samples. The results also show that bleachability of the biopretreated fabrics is significantly better than that of the conventionally scoured sample. Colour evenness of the bleached fabrics is 0.2 or below, so all the treated fabrics are homogeneous in colour.

It can be summarised that application of a hydrogen peroxide bleaching step subsequent to bioscouring is beneficial, because it reduces the large colour differences that exist between the conventionally scoured and biopretreated cotton fabric samples.

4.1.3. Dyeability with reactive dye (II)

Insufficient preparation can cause uneven dyeing, therefore careful pretreatment before dyeing is very important. It should ensure that the substrate has a high and uniform dye uptake and absorbency (Hickman, 1995). Although our results prove also that absorbency and colour evenness of the fabrics pretreated with enzyme are good enough and equal to those of the conventionally scoured fabric, it is worthwhile to characterise the dyeing behaviour of the biopretreated fabrics, and to compare their colour with that of the alkaline scoured and dyed fabric in detail. Elucidation of the effect of the subsequent bleaching step on the dyeing behaviour of the biopretreated fabrics is also very important.

Figures 7a and b illustrate the colour differences at 0.2 and 2 % dye concentrations, respectively. It is obvious from the data that without bleaching, the colour differences that exist between the alkaline scoured and bioscoured samples after dyeing are significantly greater than those for the dyed fabrics, which are bleached after the pretreatment. Data also show that colour differences depend largely on dye concentration. The
4. Results and Discussion

Figure 7. Colour differences between bioscourd and conventionally scoured cotton fabrics after reactive dyeing. Effect of dye concentration (a) 0.2 %, b) 2 %) and bleaching.
higher the dye concentration, the lower the colour difference between the alkaline scoured and bioscoured samples after dyeing.

Visual evaluation of the dyed fabrics shows that without bleaching, the colour differences at the dye concentrations of 0.2 and 0.5% are perceptible and too great to be acceptable. However, at higher dye concentrations (i.e., 1 and 2%), despite the measured higher colour difference values, the bioscoured samples conform to the conventionally scoured and dyed sample. Hydrogen peroxide bleaching between pretreatment and dyeing, at all the dye concentrations investigated, yields colour differences that are not perceptible, and the pretreated samples match the alkaline scoured sample.

All the dyed samples (with or without bleaching) appear homogeneous in colour and free from any defects related to dyeing process. Most of the $\Delta E_{ab}^*$ values are lower than 0.5, which is considered to be a visually unnoticeable colour difference. We can therefore summarise that biopretreatment results in homogeneously scoured fabrics that can be dyed uniformly. This means also that enzyme treated samples are ready for subsequent dyeing without further oxidative bleaching.

Even a perfect dyeing of a biopretreated sample may be rendered worthless if the fastness properties of the fabrics are poor. The results show that wash fastness values of the enzyme treated and conventionally scoured fabrics, with or without application of a bleaching step, do not differ and are optimal (grade 5) both for staining and colour change. Wash fastness tests cannot determine differences between samples pretreated in different ways, or those bleached prior to dyeing.

### 4.2. Bioscouring with EDTA addition

Both in bioscouring of cotton and enzyme-retting of flax, degradation of pectic substances is the most essential process. Pectin acts as a glue in the primary wall of cotton, keeping together the noncellulosic components, and also in flax stems, where pectin fixes the bast fibres to the epidermis and to the core as well. Pectin interacts itself forming calcium cross bridges, which link the pectin components to one another. The calcium ion can bind pectin and other polysaccharides too. Consequently, removal of calcium ions can accelerate the degradation of calcium-rich pectic substances.
Application of chelating substances to desiccated flax to enhance microbial-retting was first reported several years ago (Sharma, 1986). Comprehensive research works on enzyme retting of flax have shown that among several chelators tested, ethylenediaminetetraacetic acid (EDTA) is the most efficient chelator in improving enzyme retting of flax (Akin et al., 1998, 2002; Adamsen et al., 2002; Henriksson et al., 1997). Our initial assumption was that chelators could accelerate the removal of the noncellulosic components of cotton fibre. It has been demonstrated recently that metal ion content of cotton seed-coat fragments – the most resistant impurities of cotton fabric – is extremely high comparing to that of the cotton fibre itself (I). According to ICP-OES determinations the amount of calcium ions in loose cotton fibre is about 500 ppm, whereas in seed-coat fragments is more than 25000 ppm. Therefore the enzymatic degradation of cotton seed-coat fragments can also be accelerated by chelating.

4.2.1. Characteristics of cotton fabric (I)

Biopreparation of desized cotton fabrics with different enzymes for 1 hour was investigated. The weight loss values of cotton fabrics are in the range of 0–3.0 %. Celluclast 1.5L, which exhibited high filter paper activity, causes the highest cotton cellulose degradation. Except for this enzyme, agitation has only a slight effect on the enzyme action. Conventionally scouring gives a significantly greater weight loss, 5.6 %. Addition of EDTA to the enzyme solution results in higher weight losses of the cotton fabric. The small weight loss values caused by the enzymes show that the noncellulosic constituents of the cuticle are removed without significant cotton cellulose degradation.

Desized cotton fabric has an averaged lightness value of 87.5. The same value for conventionally scoured fabric is 90.8. Biopreparation with different enzymes with or without EDTA addition has only a slight effect on fabric lightness.

4.2.2. Seed-coat fragment degradation (I)

Biopreparation of cotton seed-coat fragments has been carried out similarly to the treatment of desized cotton fabric. Degradation of the impurities is characterised by weight loss. Figure 8 shows that treatment of seed-coat fragments with different enzymes results in significant weight loss. The treating solutions has become brown, indicating that some chromophores from the impurity have been released. The seed-coat fragment degradation can be enhanced significantly by EDTA addition. The most remarkable degradation effect is observed when EDTA is combined
with Pulpzyme HC. The seed-coat fragment degrading ability can only be slightly enhanced by agitation. Based upon these results it can be stated that addition of a chelator to the enzymes accelerates the degradation of the seed-coat fragment samples.

It has been demonstrated earlier (Csiszár et al., 1998) that seed-coat fragment samples turned to dark brown in conventional alkaline scouring. However, if cellulase pretreatment is used before conventionally scouring, the combined treatment resulted in light brown seed-coat fragments. In this study we have characterised the effect of enzymatic treatments on changes in colour of seed-coat fragments quantitatively. Untreated sample has an averaged lightness value of 44.7. Pretreatments in slightly acidic solution (Celluclast 1.5L, Viscozyme 120L) increase the lightness of the impurities (Figure 9), contrary to the sample treated in neutral medium (Pulpzyme HC). In neutral medium lightness value decreases by about 4–7 units. EDTA enhances the increase of lightness at pH 5, and weakens the decrease of lightness at pH 7. In agitated solutions higher lightness values are measured than in nonagitated ones.

Figure 8. Effect of chelating agent addition on the weight loss of seed-coat fragments in agitated system.
4.3. Role of EDTA in bioscouring of cellulosics

Results in the previous chapter show that EDTA improves the effectiveness of commercial enzymes, as well as accelerates both the removal of the impurities from cotton fabric and the degradation of seed-coat fragments separated from the fabric. In this chapter it is explored how the EDTA can improve the efficiency of the enzyme process in bioscouring. The effects of EDTA on the apparent activities of commercial pectinase and xylanase enzymes, and on the degradation of non-cellulosic constituents in bioscouring are investigated in detail.

4.3.1. Effect of EDTA on enzyme activity (III)

Viscozyme 120L is a pectinase rich enzyme with significant 1,4-β-endoglucanase activity (Table 3). The enzyme complex shows negligible xylanase, cellulase and β-glucosidase activities too. Pulpzyme HC is almost a pure xylanase enzyme with practically zero cellulase (FPA), β-glucosidase and pectinase activities under standard assay conditions; a very small amount of 1,4-β-endoglucanase activity can be detected (Table 3). Influence of EDTA on the enzyme activities of the two enzymes was investigated in more detail. EDTA with concentrations of 0–8.6 mM/ml enzyme was added to the enzyme solution and the standard activity assays were performed.

Figure 9. Effect of chelating agent addition on the lightness of seed-coat fragments. Data based on the untreated sample.
Table 3. Activities of commercial enzymes used throughout the experiments. 

<table>
<thead>
<tr>
<th>Enzymes</th>
<th>Cellulase (FPA) [FPU/ml]</th>
<th>1,4-β-endo-glucanase [EGU/ml]</th>
<th>β-glucosidase [IU/ml]</th>
<th>Pectinase [IU/ml]</th>
<th>Xylanase [IU/ml]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Celluclast 1.5L</td>
<td>61 (0.2)</td>
<td>22700 (560)</td>
<td>14 (0.2)</td>
<td>114 (6)</td>
<td>417 (7)</td>
</tr>
<tr>
<td>Viscozyme 120L</td>
<td>2 (0.3)</td>
<td>8600 (80)</td>
<td>4 (0.2)</td>
<td>7900 (380)</td>
<td>110 (3)</td>
</tr>
<tr>
<td>Pulpzyme HC</td>
<td>0 (0.8)</td>
<td>50 (0.8)</td>
<td>0</td>
<td>0</td>
<td>13800 (550)</td>
</tr>
</tbody>
</table>

* Standard deviations in brackets.

The influence of EDTA on the main activities of the enzymes investigated is shown in Figure 10 and 11. For Viscozyme 120L, the pectinase activity (Figure 10) are only slightly inhibited by EDTA similarly to the 1,4-β-endoglucanase and xylanase activities. On the other hand, EDTA has no effect on cellulase (FPA) and β-glucosidase activities.

For Pulpzyme HC, EDTA does not inhibit the xylanase activity significantly (Figure 11). Although the data vary near the initial activity value with the EDTA concentration, but no trend can be observed. EDTA causes measurable decrease in 1,4-β-endoglucanase activity.

It can be summarised from the results that EDTA does not inhibit significantly the main activities of the enzymes investigated, thus pectinase activity of Viscozyme 120L and xylanase activity of Pulpzyme HC retain almost unchanged in the presence of EDTA.

There are many so-called metallo-enzymes that contain metal ion in their structure (Kobayashi et al., 1999, 2001; Sakamoto et al., 1994; Spurway et al., 1997). By removing the metal ion by chelating, tertiary structure of the enzyme can be destroyed. As a consequence, the activity may decrease or disappear. Analysis of the two enzymes used in this research by ICP-OES method show that they contain approximately equal amounts of phosphorus, and magnesium. For Viscozyme 120L, the amount of sodium and potassium ions, and for Pulpzyme HC, the amount of calcium ion is significant.

4.3. Role of EDTA in bioscouring of cellulosics
4. Results and Discussion

Figure 10. Effect of EDTA on pectinase activity of Viscozyme 120L enzyme.

Figure 11. Effect of EDTA on xylanase activity of Pulpzyme HC enzyme.
Our results prove that there is no significant activity loss in the presence of EDTA if Viscozyme 120L or Pulpzyme HC enzymes are used together with EDTA. Thus, these metal ions are not part of the enzyme structure, and EDTA does not change the tertiary structure of the enzymes by chelating. The origin of metal ions in the commercial enzymes may be attributed to the medium ingredients used in fermentation and to the additives during the downstream processing.

4.3.2. Effect of EDTA on seed-coat fragment degradation (III) and linen properties (IV)

Seed-coat fragment samples have been subjected to different treatments, i.e. EDTA-only, enzyme-only, enzyme subsequent to the EDTA pretreatment and co-application of enzyme and EDTA in one bath. The overall degradation of the solid materials was characterised by the weight loss (Table 4). While the weight loss values for the two enzyme treatments are essentially the same, the pectinase treatment in acidic buffer decreases the calcium content enormously, while the xylanase treatment at pH 7 causes only a milder decrease. For EDTA + Pulpzyme HC two-step treatment, the effect of EDTA is more remarkable than the action of enzyme.

Table 4. Effect of EDTA on cotton bioscouring. Degradation of cotton seed-coat fragments: weight loss and residual calcium ion content.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Weight loss [%]</th>
<th>Calcium ion [ppm]</th>
</tr>
</thead>
<tbody>
<tr>
<td>- (untreated)</td>
<td>---</td>
<td>34900</td>
</tr>
<tr>
<td>EDTA *</td>
<td>10.6</td>
<td>18500</td>
</tr>
<tr>
<td>Viscozyme 120L *</td>
<td>14.8</td>
<td>17700</td>
</tr>
<tr>
<td>EDTA + Viscozyme 120L *</td>
<td>22.5</td>
<td>13200</td>
</tr>
<tr>
<td>Viscozyme 120L with EDTA *</td>
<td>21.5</td>
<td>11900</td>
</tr>
<tr>
<td>Pulpzyme HC *</td>
<td>14.1</td>
<td>31100</td>
</tr>
<tr>
<td>EDTA + Pulpzyme HC *</td>
<td>25.0</td>
<td>16000</td>
</tr>
<tr>
<td>Pulpzyme HC with EDTA *</td>
<td>22.2</td>
<td>16800</td>
</tr>
</tbody>
</table>

*a Treatment with EDTA. * Treatment with enzyme. * Pretreatment with EDTA and subsequent enzyme treatment. * Enzyme treatment with EDTA.

While weight loss data characterised the overall degradation effect of the applied treatments, reducing sugars in the treatment filtrates reveal the ef-
The effectiveness of the enzyme action. As Figures 12 shows, the EDTA pretreatment decreases significantly the hydrolysis level in the subsequent enzyme treatment, indicating changes in substrate structure by extraction of the divalent ions. Removal of the calcium cross bridges by EDTA pretreatment can cause a temporarily open structure, which can collapse by the end of the pretreatment, resulting in a less accessible structure for the enzyme macromolecules applied subsequently. On the other hand, addition of EDTA into the enzyme solution can accelerate the hydrolysis degree enormously (Figures 12). Removal of calcium ions from calcium cross-bridges in hemicelluloses by EDTA results in somewhat free and accessible areas in the substrate where the enzyme present is prompt to act in catalysis of chain-cutting process. Thus, in this case the enzymes are capable of accessing the macromolecules released in the simultaneous chelating process.

Taken altogether, EDTA has significant effect on the efficiency of the enzymes i.e. pectinase and xylanase, since in both pectin and xylan polymers there are divalent ion cross-bridges. Their removal modifies the polymer structure and alters its accessibility for the enzymes.

We have also investigated the ‘EDTA-enzyme-substrate’ interaction with an additional substrate, namely all-linen fabric, for confirming the results
noted above. The pectin-rich linen contains only 70 % of cellulose, and a high portion of noncellulosic matter. The influence of EDTA on the enzyme performance, as well as on the degradation of the noncellulosic con-
stituents in biopreparation was investigated in detail. The main goal of the study was the comparison of the degradation efficiency of the noncellulosic constituents of linen in biopreparation with or without EDTA.

Results prove that adding EDTA into the enzyme solution accelerates the degree of hydrolysis, and results in a higher reducing sugar liberation and more efficient calcium ion extraction, indicating a synergetic effect of enzyme and EDTA in one treating bath. However, when EDTA is applied as a pretreatment, a decrease in the efficiency of the subsequent enzymatic hydrolysis is observed (Figure 13 and 14). These results confirm our earlier findings in the field of cotton biopreparation that EDTA modifies the substrate structure by removing the calcium ions from the cross-bridges of pectin.
5. CONCLUSIONS

Biotechnology offers a wide range of alternative environmentally-friendly processes for the textile industry to complement or improve the conventional technologies. Biopreparation of the cellulosic fibres is an enzyme-aided process by which the noncellulosic ‘impurities’ (i.e. waxes, pectic substances, proteins, lignin-containing and colouring materials, etc.) are removed mainly by pectinase rich enzymes. Enzymes act under mild conditions (pH, temperature) with low water consumption; while the conventional alkaline scouring carried out with hot caustic soda is unquestionably an energy, water and chemical-intensive process. Thus, bioscouring provides an environmentally friendly alternative to the conventional alkaline process.

This study has been among the first attempts to study the effects of different enzymes with a chelator, and their synergetic action on biopreparation of cotton fabric. The results obtained provide new information on the effects of commercial enzymes on the properties of cotton and linen fabrics and degradation of cotton seed-coat fragments, as well as on better understanding of the mechanism of the chelator-aided biopreparation.

The most important conclusions of this study can be briefly summarised in the following main points:

1. Besides cellulases and pectinases, xylanase enzyme can also be used effectively in biopreparation of cotton fabric. Biopretreatment results in hydrophilic and homogeneously absorbent fabric with excellent colour evenness.

2. Whiteness of the biopretreated fabrics is less than that of the conventionally scoured samples. However, a hydrogen peroxide bleaching applied subsequent to the biopretreatment overcomes the colour differences between conventionally scoured and bioscourved samples. Hydrogen peroxide bleaching does not cause divergences and perceptible colour differences in the samples.

3. Biopretreated fabrics can be dyed with a reactive dye subsequent to the enzyme treatment without further oxidative bleaching. At higher dye concentrations, there is no perceptible colour difference between the biopretreated and alkaline scoured fabrics in dyed state. In pale and me-
dium dyeing, however, the colour difference is great and perceptible. Bleaching applied subsequent to bioscouring significantly decreases the colour difference between the dyed samples pretreated in different ways.

4. Efficiency of the biopreparation process can be enhanced significantly by adding ethylenediaminetetraacetic acid (EDTA) chelator to the enzyme solution. EDTA accelerates the degradation of cotton seed-coat fragments, the most resistant impurities of cotton. Furthermore, EDTA improves the lightening and weakens the darkening effects of the enzyme treatments carried out in acidic or neutral medium, respectively.

5. Results on ‘enzyme-EDTA-substrate’ interaction investigated in biopreparation of cotton and linen prove that application of EDTA in different concentrations does not inhibit the main activities of the hydrolytic enzymes (pectinase, xylanase), but does not increase them either. EDTA modifies the substrate structure by removing the calcium ions from the cross-bridges, linked the macromolecules in pectin.
6. REFERENCES


6. References


Enzymes and chelating agent in cotton pretreatment

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Abstract

Desized cotton fabric and cotton seed-coat fragments (impurities) have been treated with commercial cellulase (Celluclast 1.5 L), hemicellulase–pectinase (Viscozyme 120 L) and xylanase (Pulpzyme HC) enzymes. Seed-coat fragments hydrolyzed much faster than the cotton fabric itself. This relative difference in hydrolysis rates makes possible a direct enzymatic removal of seed-coat fragments from desized cotton fabric. Addition of chelating agents such as ethylenediamine-tetra-acetic acid (EDTA) markedly enhanced the directed enzyme action. Pretreatments carried out in acidic solution at pH 5 increased the lightness of seed-coat fragments, contrary to the samples treated in neutral medium at pH 7. Alkaline scouring resulted in darker seed-coat fragments except for the samples pretreated with Pulpzyme HC plus EDTA. This effect is similar to that observed in the biobleaching process in pulp and paper industry. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Bioscouring; Cellulases; Chelating agent; Cotton; Hemicellulases; Seed-coat fragments

1. Introduction

The serious waste water pollution caused by textile finishing has oriented recent research towards application of enzymes in wet processes. Cellulases, hemicellulases and pectinases acting on native cellulosic fibres (cotton, flax, hemp, jute, etc.) became the target enzymes in bioprocessing (Lange, 1993). The production of ‘aged’ denim garments with cellulases is the most successful enzyme process that has emerged in the textile industry in the last decade. Biofinishing or biopoothing represents another application of cellulases for non-denim cellulose fabrics and garments (Cavaco-Paulo, 1998). In the environmentally-friendly process, cellulosic fabrics are subjected to a treatment with cellulolytic enzymes, and the action of cellulases removes small loose fibre ends that protrude from the fabric surface.

Recent results indicate that enzymes (mainly cellulases and several noncellulolytic enzymes such as lipases, proteases and pectinases) may be used effectively in the cleaning procedure of cotton. In the traditional scouring process—which is energy and chemical intensive—concentrated sodium hydroxide solution and additional hydrogen peroxide and/or sodium hypochlorite solutions are applied for removing the impurities from
raw cotton. The noncellulosic 'impurities' (waxes, pectins, proteins, noncellulosic polysaccharides, inorganics, lignin-containing impurities, colouring materials, etc.) in raw cotton represent approx. 10% of the total weight. The mild reaction conditions offered by enzymatic treatment provide an environmentally friendly alternative (Li and Hardin, 1997; Buchert et al., 1998; Hartzell and Hsieh, 1998).

The main goals in cotton pretreatment are to arrive at satisfactory absorbency, a complete removal of seed-coat fragments and the achievement of appropriate whiteness. It has been reported (Hartzell and Hsieh, 1998) that cellulases are the most effective enzymes in improving water wettability of cotton. The greatest obstacle to commercialisation of bioprepARATION is removal of matter (stalks, leaves and seed-coat fragments) of vegetable origin. Seed-coat fragments are part of the outer layer of cottonseed and they are formed from mature or immature seeds during mechanical processing. They are usually black or dark brown and may or may not have fibers and linters attached (Annual Book of ASTM Standards, 1980; Verschraeg, 1989). Seed-coat fragments are the most resistant impurities of cotton. Their removal requires, in the traditional chemical processes, more concentrated chemical solutions and longer steaming periods than the elimination of other impurities from raw cotton. In practice, however, the total seed-coat fragments are not removed, and the residual part is only bleached in modern preparatory processes.

We have demonstrated recently (Csiszár et al., 1996, 1998a), that cellulase treatment prior to alkaline scouring, enhanced both the removal and degradation of seed-coat fragment impurities of cotton fabrics. The tiny fibres that attached the seed-coat fragments to the fabric were hydrolysed by the enzyme. The cellulase enzyme complex made the residual seed-coat fragments more accessible to chemicals. When consecutive cellulase treatment and conventional alkaline scouring were combined, the increase in whiteness of the fabrics was more significant. Cellulase pretreatment also allowed the reduction of the hydrogen peroxide consumption in the consecutive chemical bleaching step (Csiszár et al., 1998b). The effect of seven commercial cellulase and hemicellulase enzymes on the weight loss, reducing sugar liberation and degree of polymerisation of desized cotton fabric was also studied in agitated and non-agitated conditions (Csiszár et al., 2001).

The present work—based on our earlier results—is focusing on the enzymatic degradation of cotton seed-coat fragments. Three commercial enzymes have been used throughout the experiments in agitated and non-agitated solutions. One selected enzyme (Celluloast 1.5 L) has high cellulase (filter paper) activity. The second enzyme complex (Viscozyme 120 L) possesses significant pectinase activity (Buchert et al., 1998). The third enzyme (Pulzynz HC) represents only xylanase activity. Since seed-coat fragments differ in chemical composition, colour and morphology from cotton cellulose, these three different lignocellulosic enzymes enable us to compare them for seed-coat fragment degradation and for impurity removal from crude cotton fibre.

The rates of degradation of seed-coat fragments and that of desized cotton fabric were compared. Weight loss and lightness of the substrates were also measured after the enzymatic and the subsequent alkaline treatments. Because our earlier results indicated that the metal ion content of the seed-coat fragments was extremely high compared to that of the cotton fibre (Csiszár et al., 1987), the metal ion content of the substrates and the influence of chelating agents on that, as well as on the enzyme action, were studied.

2. Materials and methods

Greige cotton print cloth fabric (122 gm−2) obtained from Testfabric Inc., NJ was used for the experiments after amylase enzymatic desizing. Spinning blowroom waste (a combined waste produced during the mechanical steps of cotton purification) was selected to model the impurities. It consists of stalks, leaves and seed-coat fragments of the cotton (Csiszár et al., 1998a). The average size of the seed-coat fragments parallel to the surface of the raw cotton fabric was 0.67 mm, the value measured in the perpendicular direction was 0.25 mm. The fraction studied in this work
was composed of particles between the diameters of 0.32 and 0.64 mm. Celluclast 1.5 L (Trichoderma reesei origin), Viscozyme 120 L (Aspergillus sp. origin) and Pulpzyme HC (Bacillus sp. origin) enzymes were kindly supplied by Novo-Nordisk A/S, Copenhagen, Denmark.

Spinning blowroom waste (5 g) and desized cotton fabric (3 g) were treated with enzymes in non-agitated and agitated systems, respectively, at pH 5.0 (0.05 M acetate buffer) for the enzymes Celluclast 1.5 L and Viscozyme 120 L, or at pH 7.0 (0.05 M phosphate buffer) for the enzyme Pulpzyme HC; a liquor ratio of 1:100; enzyme concentration of 1 g l\(^{-1}\); treatment times of 0.5, 1, 1.5 and 2 h; incubation temperature of 50 °C; and a nonionic surfactant concentration of 1 g l\(^{-1}\). In the agitated system the enzymatic treatment was accomplished in a Scourotester laboratory unit. The agitation speed was set to 40 ± 2 rpm during the biotreatment. When the effect of chelating agents was tested, 0.5 mmol ethylenediaminetetra-acetic acid (EDTA) g\(^{-1}\) substrate was added to the enzyme solution. After enzymatic treatment the substrate was washed twice in hot distilled water to deactivate the enzyme and then air-dried. Reference treatments were carried out similarly without enzyme addition.

Caustic scouring was performed with the pad-steam method at 100 °C for 20 min in a solution containing 50 g l\(^{-1}\) sodium hydroxide and 1 g l\(^{-1}\) surfactant.

Weight loss was determined by weighing the samples before and after the treatment following 24 h of conditioning at 20 °C and 65% rh.

Changes in colour of seed-coat fragment and cotton fabric were characterised by the lightness (L*\(_{ab}\)) values. The seed-coat fragment samples were milled and pressed to standard pastilles after enzymatic and subsequent alkaline treatments, enabling the reproducible registration of their colour. X, Y, Z tristimulus values of the pastilles and fabrics in CIELAB colour space were measured with a Hunterlab Color QUEST (D65/10°) colour-measuring instrument after enzymatic and consecutive enzymatic and alkaline scouring processes. Lightness values were calculated from the corresponding data.

The calcium ion content of the seed-coat fragment samples pretreated with different enzymes for 1 h, was determined by the inductively coupled plasma optical emission method (ICP-OES). Dry sample (0.8 g) was measured into the inserts of the PTFE bomb. High purity nitric acid (6 ml; 65% Reanal a.r. purified by subboiling distillation) and 2 ml of hydrogen peroxide (30%, Reanal a.r.) was added. After 3 h digestion at room temperature the insert was placed into the PTFE bomb and the sample was digested for 4 h at 130 °C. The solution was transferred into a 25 ml volumetric flask, then the sample solution was filtered into a dry PE container. The sample solution was diluted ten-times for the measurement of calcium and magnesium. The element concentrations of the diluted and undiluted sample solutions were measured by the ICP-OES method in simultaneous multi-element mode by a 40 channel Labtest Plasmalab ICP-spectrometer using 27 MHz Ar–Ar plasma of 1.3 kW, 13 mm of observation height. The sample introduction was made by GMK V-groove nebuliser.

3. Results and discussion

3.1. Enzymatic-chelating degradation of cotton seed-coat fragments and desized cotton fabric

The treatment of seed-coat fragments (spinning blowroom waste) in buffer at 50 °C for 30 min surprisingly resulted in significant weight loss (Fig. 1). The treating solutions became brown, indicating that some chromophores from the impurity have been released. The weight loss, however, could be increased by the addition of enzyme, chelating agent (EDTA) or both. Interestingly, at higher pH (7.0) EDTA was effective alone in buffer, but not at a lower pH (5.0). The most remarkable degradation effect was observed when EDTA was combined with Pulpzyme HC. Our results support earlier observations that EDTA has a positive effect on the microbial retting of flax (Brown and Black, 1985) or on enzymatic retting of flax (Henriksson et al., 1997). The seed-coat fragment degrading ability of the enzymes could only be slightly enhanced by agita-
Appendix I.

Fig. 1. Effect of time and chelating agent addition on the weight loss of seed-coat fragments in nonagitated solution with or without enzymes.

Table 1
Weight loss values of desized cotton fabrics during treatments (1 h) with different enzymes: effect of agitation and chelating agent addition

<table>
<thead>
<tr>
<th>Mode of pretreatment</th>
<th>Weight loss (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Nonagitated</td>
</tr>
<tr>
<td>Buffer (pH 5)</td>
<td>0</td>
</tr>
<tr>
<td>Buffer (pH 5)+ EDTA</td>
<td>1.0 ± 0.2</td>
</tr>
<tr>
<td>Celluclast 1.5 L</td>
<td>1.4 ± 0.1</td>
</tr>
<tr>
<td>Celluclast 1.5 L + EDTA</td>
<td>2.1 ± 0.2</td>
</tr>
<tr>
<td>Viscozyme 120 L</td>
<td>1.5 ± 0.1</td>
</tr>
<tr>
<td>Viscozyme 120 L + EDTA</td>
<td>1.5 ± 0.2</td>
</tr>
<tr>
<td>Buffer (pH 7)</td>
<td>0.4 ± 0.1</td>
</tr>
<tr>
<td>Buffer (pH 7)+ EDTA</td>
<td>0.8 ± 0.1</td>
</tr>
<tr>
<td>Pulpzyme HC</td>
<td>0.4 ± 0.1</td>
</tr>
<tr>
<td>Pulpzyme HC + EDTA</td>
<td>1.3 ± 0.1</td>
</tr>
</tbody>
</table>

For example, in agitated solutions, Pulpzyme HC coupled with EDTA resulted in an additional 1–3% weight loss.

Treatments with the same enzymes for 1 h caused much lower degradation in cotton fabric than for seed-coat fragments (Table 1). The weight loss values measured are in the range of 0–3.0%. Celluclast 1.5 L, which exhibited high filter paper activity, caused the highest cotton cellulose degradation. Except for this enzyme, agitation has only a slight effect on the enzyme action characterised by weight loss. Addition of EDTA to the enzyme solution resulted in higher weight losses of the cotton fabric. The small weight loss values caused by the enzymes showed that the noncellulosic constituents of the cuticle were removed without significant cotton cellulose degradation. Based upon these results it could be stated that addition of a chelator to the buffers accelerated both the removal of the impurities from the cotton fabric, and the degradation of the seed-coat fragment samples.

3.2. Alkaline degradation of seed-coat fragments and desized cotton fabric after the enzymatic-chelating pretreatments

Subsequent to the different pretreatments (1 h) the residual seed-coat fragments and the cotton fabrics were scoured with caustic soda solution. Though different pretreatments solubilized approximately 30% of the seed-coat fragments (Fig. 1), considerable additional degradation has been observed during the subsequent scouring process (see Table 2). A 65.9% weight loss value was found when the seed-coat fragment was not pre-treated, but only directly scoured. Depending on the conditions of the pretreatments the weight loss could be enhanced up to approx. 72%. Pretreatment with either Viscozyme 120 L or Pulpzyme HC enzymes combined with EDTA caused the highest degradation of seed-coat fragments during the subsequent alkaline treatment. The presence of EDTA in the pretreating solution had a significant effect on the subsequent alkaline degradation of the seed-coat fragment samples. Agitation of the pretreating solution did not remarkably increase the alkaline degradation of the impurities.
Enzymes and chelating agent in cotton pretreatment

Table 2
Weight loss values of seed-coat fragments and sized cotton fabrics during alkaline scouring subsequent to different pretreatments

<table>
<thead>
<tr>
<th>Mode of pretreatment</th>
<th>Weight loss of seed-coat fragment (%)</th>
<th>Weight loss of cotton fabric (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Non agitated</td>
<td>Agitated</td>
</tr>
<tr>
<td>No pretreatment (only alkaline scouring)</td>
<td>65.9 ± 1.2</td>
<td>60.3 ± 1.1</td>
</tr>
<tr>
<td>Buffer (pH 5)</td>
<td>60.0 ± 0.4</td>
<td>(70.3)</td>
</tr>
<tr>
<td></td>
<td>(70.2)*</td>
<td>(5.6)</td>
</tr>
<tr>
<td>Buffer (pH 5)+EDTA</td>
<td>66.8 ± 0.7</td>
<td>67.7 ± 1.8</td>
</tr>
<tr>
<td></td>
<td>(75.1)</td>
<td>(75.8)</td>
</tr>
<tr>
<td>Celluclast 1.5 L</td>
<td>61.6 ± 2.0</td>
<td>61.8 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>(71.6)</td>
<td>(71.7)</td>
</tr>
<tr>
<td>Celluclast 1.5 L+EDTA</td>
<td>67.6 ± 1.1</td>
<td>69.5 ± 0.4</td>
</tr>
<tr>
<td></td>
<td>(76.3)</td>
<td>(77.9)</td>
</tr>
<tr>
<td>Viscozyme 120 L</td>
<td>62.3 ± 2.2</td>
<td>62.1 ± 0.7</td>
</tr>
<tr>
<td></td>
<td>(71.9)</td>
<td>(71.8)</td>
</tr>
<tr>
<td>Viscozyme 120 L+EDTA</td>
<td>69.5 ± 1.6</td>
<td>72.3 ± 1.0</td>
</tr>
<tr>
<td></td>
<td>(77.9)</td>
<td>(80.1)</td>
</tr>
<tr>
<td>Buffer (pH 7)</td>
<td>65.8 ± 0.9</td>
<td>66.7 ± 1.0</td>
</tr>
<tr>
<td></td>
<td>(74.4)</td>
<td>(75.1)</td>
</tr>
<tr>
<td>Buffer (pH 7)+EDTA</td>
<td>67.0 ± 1.2</td>
<td>69.0 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>(76.2)</td>
<td>(78.0)</td>
</tr>
<tr>
<td>Pulpzyme HC</td>
<td>67.2 ± 1.4</td>
<td>67.4 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>(75.3)</td>
<td>(75.5)</td>
</tr>
<tr>
<td>Pulpzyme HC+EDTA</td>
<td>67.7 ± 1.0</td>
<td>70.4 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>(77.6)</td>
<td>(79.7)</td>
</tr>
</tbody>
</table>

* Total weight loss values (calculated): weight loss during pretreatment plus weight loss in alkaline scouring.

With consecutive enzymatic treatment and alkaline scouring, weight losses were around 80% for the best combinations (see values in brackets in Table 2).

Alkaline scouring of sized cotton fabric caused 5.6% weight loss. None of the pretreatments remarkably enhanced the effect of sodium hydroxide on the cotton fabric. Scouring did not cause a greater weight loss subsequent to the pretreatments. The weight loss values measured in the range of 4.5–6.2%; they are around the generally accepted limit, and any trend or correlation could not be observed. Consequently, under the conditions studied, pretreatments did not make the cotton fabrics more accessible to scouring chemicals. With consecutive enzymatic and alkaline treatments, total weight loss was as high as 5.1–7.9% (see values in brackets in Table 2). The highest weight loss (7.9%) was observed when Celluclast 1.5 L and EDTA were used for pretreatment.

Viscozyme 120 L or Pulpzyme HC with EDTA was the most effective pretreatment in seed-coat fragment degradation and none of them caused increased cotton cellulose damage. Thus, selective degradation of seed-coat fragments can be accelerated by pretreatment with xylanase or pectinase enzymes combined with EDTA.

### 3.3. Changes in calcium ion content of seed-coat fragments during the enzymatic-chelating pretreatments

According to ICP-OES determinations the metal ion content in seed-coat fragments was much higher than in cotton fibre. For example, the calcium ion content in loose cotton fibre was about 500 ppm, whereas in seed-coat fragments more than 25 000 ppm. Pectin substances in plants usually form aggregates with calcium ions that crosslink pectin chains to each other (Henriksson et al., 1997). Therefore, the decrease of the calcium content may promote the degradation of seed-coat fragments.

The residual calcium ion content of the seed-coat fragment samples after pretreatment is
shown in Fig. 2. Agitation facilitated the removal of calcium ions from the impurity. The samples treated in the agitated solution had larger decreases in calcium ion content (by 2–16%) than those treated in the nonagitated bath.

The phosphate buffer at pH 7 had little effect on the calcium ion content of seed-coat fragment samples, only about 5% of them were removed from the substrates, whereas, in mild acidic medium (pH 5) in the acetate buffer, 20–25% of calcium ions were dissolved. EDTA had an enormous effect in phosphate buffer, the amount of calcium ions decreased by approximately half. In acetate buffer the effect of EDTA addition was smaller.

Residual calcium ion values were almost identical for the samples treated with the enzymes, compared to the respective buffer treated controls. The combined effect of Pulzyme HC and EDTA resulted in the lowest residual calcium ion content (35% in the agitated and 49% in the nonagitated bath). There was no significant difference in the calcium ion content between Celluclast 1.5 L + EDTA and Viscozyme 120 L + EDTA treated samples, the calcium ion content decreased to 46% in the agitated solution and to about 60% in the nonagitated bath.

Based upon these results it could be concluded that at pH 7 the effect of EDTA on the removal of the calcium ion content of the seed-coat fragment samples was significant, and that of the enzyme was only slight. However, at pH 5 the effect of the enzymes was remarkable and that of EDTA was obscure. The extent of complex formation with EDTA is greatly affected by pH. At low pH values EDTA becomes less dissociated and hence the concentrations of the complexing species are seriously decreased. For calcium ions the complexing efficiency falls of at about pH 7 (Peters, 1967). At pH 7 the conditional formation constant of EDTA for calcium is $2.4 \times 10^7$ and the same constant at pH 5 is $1.75 \times 10^4$ (Skoog et al., 1988). Therefore, at pH 7 in phosphate buffer the calcium chelating power of EDTA is much higher than that at pH 5 in acetate buffer.

In a mild acid medium the synergetic action of enzymes and EDTA was obvious. The enzymes hydrolysed the natural polymers in the substrate, loosened its structure and assisted the action of EDTA by increasing the accessibility of the metal ion containing constituents. Chelator could increase the apparent enzyme activity.

A comparison of Figs. 1 and 2 revealed that the removal of calcium ions could facilitate the degradation of the seed-coat fragments. The most significant degradation occurred when elimination of calcium ions was the greatest. Calcium ions act as bridges between pectin molecules stabilising the

![Graph showing residual calcium ion percentage for different treatments](image-url)

**Fig. 2.** Decrease in calcium ion content of cotton seed-coat fragments by different pretreatments (1 h).
Enzymes and chelating agent in cotton pretreatment

3.4. Influence of enzymatic-chelating pretreatments and subsequent alkaline scouring on colour of seed-coat fragments and desized cotton fabric

We have observed earlier (Csiszár et al., 1998a), that seed-coat fragment samples turned to dark brown in conventional alkaline scouring. However, if cellulase pretreatment was used before alkaline scouring, the combined treatment resulted in light brown seed-coat fragments. In this paper we have quantitatively characterised the effect of enzymatic and alkaline treatments on changes in colour of seed-coat fragments.

Untreated sample had an averaged lightness value of 44.7 (Fig. 3). Most pretreatments in slightly acidic solution (pH 5) increased the lightness of the impurities, contrary to the samples treated in neutral medium (pH 7). In agitated solutions higher lightness values were measured than in nonagitated ones. In acidic medium the highest increase in $L_\text{ab}^*$ values was achieved by the combined effect of enzyme and chelating agent. In neutral medium lightness values decreased by about 4–7 units. EDTA tempered the darkening of the samples.

Except for two samples pretreated with Pulzyme HC + EDTA and Buffer pH 7 + EDTA respectively, alkaline treatment resulted in darkening of the seed-coat fragment impurities (Table 3). The most significant decrease in lightness was observed for the untreated sample, where the $L_\text{ab}^*$ value of 44.7 was reduced to 28.4. Pretreatments at pH 5 weakened the darkening effect of alkaline scouring. Acetate buffer applied alone resulted in a lighter sample after alkaline scouring, and this

<table>
<thead>
<tr>
<th>Mode of pretreatment</th>
<th>Lightness $[L_\text{ab}^*]$</th>
<th>Pretreated but not scoured</th>
<th>Pretreated and scoured</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated (control)</td>
<td>44.7 ± 0.9</td>
<td>28.4 ± 0.6</td>
<td></td>
</tr>
<tr>
<td>Buffer (pH 5)</td>
<td>46.1 ± 0.5</td>
<td>34.3 ± 0.9</td>
<td></td>
</tr>
<tr>
<td>Buffer (pH 5) + EDTA</td>
<td>44.8 ± 0.6</td>
<td>43.3 ± 0.4</td>
<td></td>
</tr>
<tr>
<td>Cellulase 1.5 L + EDTA</td>
<td>46.2 ± 0.9</td>
<td>38.7 ± 0.3</td>
<td></td>
</tr>
<tr>
<td>Cellulase 1.5 L + EDTA</td>
<td>48.9 ± 0.9</td>
<td>43.4 ± 0.6</td>
<td></td>
</tr>
<tr>
<td>Viscozyme 120 L + EDTA</td>
<td>46.0 ± 0.5</td>
<td>35.5 ± 0.2</td>
<td></td>
</tr>
<tr>
<td>Viscozyme 120 L + EDTA</td>
<td>47.2 ± 0.5</td>
<td>38.4 ± 0.4</td>
<td></td>
</tr>
<tr>
<td>Buffer (pH 7) + EDTA</td>
<td>38.4 ± 0.3</td>
<td>36.8 ± 0.4</td>
<td></td>
</tr>
<tr>
<td>Buffer (pH 7) + EDTA</td>
<td>40.7 ± 0.5</td>
<td>41.4 ± 0.2</td>
<td></td>
</tr>
<tr>
<td>Pulzyme HC + EDTA</td>
<td>37.4 ± 0.7</td>
<td>37.0 ± 0.4</td>
<td></td>
</tr>
<tr>
<td>Pulzyme HC + EDTA</td>
<td>40.5 ± 0.4</td>
<td>45.7 ± 0.2</td>
<td></td>
</tr>
</tbody>
</table>
Appendix I.


Fig. 4. Changes in colour of cotton fabrics during the treatments (1 h) with different enzymes. Effect of agitation and chelating agent addition on the lightness ($L_{ab}^*$).

Fig. 5. Lightness values of cotton fabrics after pretreatments (1 h) with different enzymes and subsequent alkaline scouring.

Bioleaching process with xylanases or other enzymes in pulp and paper industry (Vikari et al., 1996). Pulzyme HC applied together with EDTA modified the structure of seed-coat fragments, degraded the xylan constituents of the lignin–carbohydrate complex and removed most of the calcium ions. Enzyme action loosened the structure and enhanced the removal of lignin containing coloured impurities in the alkaline process. Elimination of calcium ions also contributed to this action.

Desized cotton fabric had an averaged lightness value of 87.5 (Fig. 4). The same value for alkaline scoured fabric was 90.8 (Fig. 5). The different pretreatments did not increase the lightness values considerably. The most efficient pretreatment was a Viscozyme 120 L and EDTA combination with a lightness value of 92.2.
Acknowledgements

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References


Bleachability and Dyeing Properties of Biopretreated and Conventionally Scoured Cotton Fabrics

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ABSTRACT

Enzymatic (cellulase, pectinase, xylanase) and simple buffer treatments in the presence of a nonionic surfactant improve water wettability of fabrics to a level equal to conventional alkaline scouring. Caustic scoured fabric is significantly lighter and less colored than all the biopretreated samples. Application of a hydrogen peroxide bleaching subsequent to the biopretreatment is beneficial because it reduces the great color differences between conventionally scoured and biopretreated samples. Bleachability of the pretreated fabrics is better than that of the conventionally scoured sample. Biopretreated fabrics can be dyed with a reactive dye subsequent to the enzymatic treatment without further oxidative bleaching. At higher dye concentrations (i.e., 1 or 2%), there is no perceptible color difference between the biopretreated and alkaline scoured fabrics in the dyed state. In pale and medium dyeings, however, the color difference is great and perceptible. Bleaching applied subsequent to bioscouring significantly decreases the color difference between the dyed samples pretreated in different ways. None of the pretreatments causes uneven dyeing. Wash fastness of the dyed samples is excellent and unrelated to the pretreatment method.

Preparation and bleaching are among the most energy- and chemical-intensive steps in traditional cotton finishing. About 75% of the organic pollutants arising from textile finishing come from the preparation of cotton goods [5, 12]. Biopreparation may therefore be a valuable and environmentally friendly alternative to harsh alkaline chemicals for preparing cotton. In recent years, different enzymes, i.e., cellulases, pectinases, lipases, and proteases, have been tested for biopreparation [1, 2, 10, 11, 14, 15]. We have demonstrated recently [3] that xylanase enzymes can also be effective in removing natural impurities from cotton.

¹To whom correspondence should be addressed: e-mail emi@muatex.mua.bme.hu
Appendix II.

After biopreparation, fabrics are generally further subjected to dyeing or printing, so it is particularly essential to characterize the dyeing properties of biopretreated substrates. Improved and uniform water wetting properties and color evenness are required for effective dyeing. Many papers have investigated how enzymes affect cotton fabric properties with respect to their effectiveness at replacing alkaline scouring and how enzymes modify the various cell wall components of cotton fibers. However, very few have reported on the bleachability and dyeing behavior of bioscouring fabrics. Eiters et al. compared the color depth [6] and color yield [7] of biopretreated and caustic scoured fabrics after direct dyeing. They obtained similar color yields with dyeings on cotton prepared by the two techniques. Traore et al. [13] reported that dyeing with direct dyes yields uniform results and comparable color strength independent of the enzyme system used. Lenting and his co-authors [9] used a basic dye to study the homogeneity of bioscouring cotton fabrics. They also compared the color of the bioscouring and alkali scoured fabrics after dyeing. Color deviations of the dyed fabrics scoured by the two different methods were substantial, and they questioned whether subsequent bleaching would overcome these deviations. None of the papers characterized the color evenness and homogeneity of bioscouring cotton fabrics with measured data or their wash fastness properties after dyeing. Furthermore, neither the bleachability of the fabric nor the effect of chemical bleaching subsequent to enzymatic treatments on the dyeing properties of the fabrics have been documented yet.

In this paper, we use a commercial cellulase, a hemi-cellulase-pectinase, and a xylanase enzyme for bioscouring desized cotton fabrics. We characterize in detail selected properties of the pretreated samples, which are important for effective dyeing. We also elucidate the effect of hydrogen peroxide bleaching, subsequent to biopretreatment, on the bleachability and dyeing properties of the samples. We use a reactive dye at four different concentrations to compare the dyeing behavior of biopretreated and biopretreated/bleached cotton fabrics with that of the alkaline scoured and alkaline scoured/bleached fabrics. We also determine the color evenness and uniformity and the wash fastness properties of the dyed samples.

Experimental

Greige cotton print cloth (style 400R, Testfabrics Inc., NJ) was desized with amylase enzyme in the presence of a nonionic surfactant before the experiments. An acidic cellulase (Celluclast 1.5 L, Trichoderma reesei origin, 67 FPU/ml), an acidic pectinase-hemicellulase (Viscozyme 120 L, Aspergillus aculeatus, 8 FPU/ml), and a cellulase-free xylanase (Pulpzyme HC, Bacillus sp., 120,000 IU/ml), gifts from Novozymes, were used for bioscouring. Further enzyme activities are detailed in a previous study [4]. We selected specific enzymes based on their different major components such as cellulase, pectinase, or xylanase.

Desized fabric samples were treated with enzymes in an agitation system (Scourtester laboratory unit, 40 ± 2 rpm) at pH 5.0 (0.05 M acetate buffer) for Celluclast 1.5 L and Viscozyme 120 L enzymes, or at pH 7.0 (0.05 M phosphate buffer) for Pulpzyme HC. The liquor ratio was 1:100, enzyme concentration was 1 g/L, treatment time was 1 hour, incubation temperature was 50°C, and the nonionic surfactant concentration was 1 g/L. After enzymatic treatment, the fabrics were washed twice in hot distilled water for 30 seconds each to deactivate the enzyme and then air-dried for 24 hours. Buffer treatments were similar but without enzyme addition in acetate or phosphate buffers in the presence of surfactant. For comparison, desized cotton fabric was scoured according to the conventional alkaline procedure in Mathis Labomat type BFA12 equipment. A 50 g/L sodium hydroxide solution was used at 95°C for 20 minutes at a liquor ratio of 1:50.

We assessed the dyeability of the fabrics by a standard dyeing method. Bioscourer, buffer treated, and conventionally scoured samples (six pieces) were dyed together at a certain dye concentration in the same dyebath in the Mathis Labomat equipment by the bath exhaustion procedure. A heterobifunctional reactive dye (Dye A, Sumitomo Co., Japan) was used at four concentrations based on the weight of the fabric. Composition of the dyebaths is shown in Table I. Fabrics were placed in the dyebath containing reactive dye and Na$_2$SO$_4$ at room temperature (fabric to liquor ratio of 1:20), and the temperature was raised slowly to 60°C within 10 minutes. After an additional 10 minutes at 60°C, the samples were taken from the dyebath and Na$_2$CO$_3$ was stirred in. Dyeing continued for another 60 minutes at 60°C after the addition of the soda. The dyed samples were washed first with cold distilled water for 5 minutes, second with a solution containing 3.5 ml/L of cc. acetic acid at 50°C for 5 minutes, then twice with distilled water at 98°C for 5 minutes, and finally once in hot tap water and three times.

<table>
<thead>
<tr>
<th>Table I. Composition of dyebaths.</th>
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<tbody>
<tr>
<td>Dye concentration, %</td>
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<tr>
<td>-----------------------</td>
</tr>
<tr>
<td>0.2</td>
</tr>
<tr>
<td>0.5</td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>2</td>
</tr>
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</table>
in cold tap water for 1 minute each. Finally, the samples were air-dried for 24 hours.

In the first dyeing experiment, conventionally scoured, bioscourd, and buffer treated fabrics were dyed directly after pretreatment. The results were compared with the findings of a second experiment, where a hydrogen peroxide bleaching step was inserted between the pretreatment and dyeing. Hydrogen peroxide bleaching occurred in the Mathis Labomat equipment at 85°C for 1 hour at a liquor ratio of 1:50 in a solution containing 2 mL/L 30% hydrogen peroxide, 1.2 g/L sodium hydroxide, and 2 mL/L stabilizer.

CHARACTERIZING THE PRETREATED, BLEACHED, AND DYED SAMPLES

Weight loss due to the pretreatments was determined by weighing the fabric samples before and after pretreatment following 24 hours of conditioning at 20°C and 65% RH. Wettability of the pretreated fabrics was characterized by the water drop test, counting the elapsed seconds between the contact of the water drop with the fabric and the disappearance of the drop into the fabric. Ten readings were taken from different locations on the sample subsequent to the pretreatment and the average was reported. Water retention values (WRV) were determined by soaking the pretreated and pretreated/bleached fabrics in distilled water for 24 hours, followed by centrifugation of 2000 g for 10 minutes and drying. WRV was calculated from the weights of a sample just after centrifugation and after drying, and expressed as the percentage of the dry weight. Three parallel determinations were made and averaged. Color was measured with a Hunterlab Color Quest (D65/10°) colorimeter and evaluated according to the CIELab color space. Each sample measurement represents the average of five readings from different positions on the swatch. Process efficiency (pretreatment, bleaching) and the divergence between the bio- and conventionally pretreated samples were characterized by the color difference (ΔE<sub>ab</sub>*) values. Color evenness of the fabrics was measured after each treatment, i.e., scouring, bleaching and dyeing. In order to evaluate color evenness, each sample was measured on ten randomly selected locations, and the X<sub>1</sub>, Y<sub>1</sub>, Z<sub>1</sub>…X<sub>10</sub> Y<sub>10</sub> Z<sub>10</sub> tristimulus values were averaged. Color difference was calculated from certain X<sub>n</sub>, Y<sub>n</sub>, Z<sub>n</sub> and the averaged X̄ Ȳ Z̄ values. Color evenness was expressed by the average of the ten color difference values (ΔE<sub>ab</sub>*). Fastness to washing of the dyed samples was tested by ISO 105-C01. Staining and color changes that occurred in wash fastness tests were assessed by means of the appropriate gray scales.

Results and Discussion

CHARACTERISTICS OF PRETREATED FABRICS

Wettability, absorbency, whiteness, and uniformity of the pretreated samples have significant effects on the success of subsequent dyeing process. Experimental data from the fabrics pretreated with different enzymes are compared with those of the buffer treated and conventionally scoured fabrics in Table II.

Greige cotton fabric is hydrophobic and can be characterized by a wetting time of more than 60 seconds. Desized cotton fabric is also hydrophobic, but it has a measurable wetting time near 10 seconds (Table II), owing to the desizing process with amylase and surfactant. All fabrics treated by enzyme and buffer are hydrophilic, and each has a wetting time of less than 1 second. Thus, the water absorbency test does not reveal significant differences between the pretreatments. Both acetate (pH 5) and phosphate (pH 7) buffers supplemented with nonionic surfactant significantly improve the hydrophilicity of the fiber surfaces. Preliminary experiments in our previous work indicated that the nonionic surfactant

<table>
<thead>
<tr>
<th>Treatments&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Wetting time, seconds</th>
<th>WRV&lt;sup&gt;b&lt;/sup&gt;, %</th>
<th>Weight loss, %</th>
<th>ΔE&lt;sub&gt;ab&lt;/sub&gt;*</th>
<th>ΔL*</th>
<th>ΔC&lt;sub&gt;ab&lt;/sub&gt;*</th>
<th>ΔH&lt;sub&gt;ab&lt;/sub&gt;*</th>
<th>ΔL*</th>
<th>ΔC&lt;sub&gt;ab&lt;/sub&gt;*</th>
<th>ΔH&lt;sub&gt;ab&lt;/sub&gt;*</th>
</tr>
</thead>
<tbody>
<tr>
<td>D</td>
<td>~10</td>
<td>36.4 (0.1)</td>
<td>ND&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.1</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>CS</td>
<td>&lt;1</td>
<td>41.1 (1.0)</td>
<td>5.2</td>
<td>0.2</td>
<td>8.4</td>
<td>6.2</td>
<td>–6.5</td>
<td>0.6</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>pH 5</td>
<td>&lt;1</td>
<td>35.0 (0.4)</td>
<td>0</td>
<td>0.1</td>
<td>0.4</td>
<td>–0.4</td>
<td>0.1</td>
<td>–0.2</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>C</td>
<td>&lt;1</td>
<td>38.0 (0.7)</td>
<td>0.8</td>
<td>0.2</td>
<td>0.7</td>
<td>0.6</td>
<td>–0.3</td>
<td>–0.0</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>V</td>
<td>&lt;1</td>
<td>36.5 (0.7)</td>
<td>0</td>
<td>0.2</td>
<td>0.4</td>
<td>0.4</td>
<td>–0.1</td>
<td>–0.0</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>pH 7</td>
<td>&lt;1</td>
<td>42.3 (0.1)</td>
<td>0.2</td>
<td>0.2</td>
<td>1.2</td>
<td>1.2</td>
<td>–0.4</td>
<td>0.0</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>P</td>
<td>&lt;1</td>
<td>42.1 (1.1)</td>
<td>0</td>
<td>0.2</td>
<td>1.1</td>
<td>1.1</td>
<td>–0.4</td>
<td>0.0</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

<sup>a</sup> Abbreviations of the treatments: D = desizing, CS = conventional scouring, pH 5 and pH 7 = buffer with surfactant, C = Celluclast 1.5 L, V = Viscozyme 120 L, P = Pulpzyme HC.  
<sup>b</sup> Standard deviations in brackets.  
<sup>c</sup> Color evenness.  
<sup>d</sup> Not determined or calculated.  
<sup>*</sup> ΔE<sub>ab</sub>* values are shown in Figure 1 (A).
did not inhibit the activity of the enzymes. Our results confirm our earlier findings that the surfactant is highly effective at improving the wetting and absorbency of the desized cotton fabric. Additionally, averaged values of wetting time calculated from ten determinations from varying locations on the samples subsequent to the pretreatment show that none of the readings on randomly selected locations indicated an inhomogeneous fabric surface and non-uniform absorbency. We can state that both the enzymatic and simple buffer treatments in the presence of an appropriate surfactant improve water wettability of the cotton fabric to a level adequate for dyeing, printing, and finishing, and they result in homogeneously absorbent fabrics. Water absorbency achieved with or without enzyme is sufficient and equal to that obtained by conventional scouring.

Water retention values (WRV, Table II) show that the hydrophobic surface of the desized fabric results in low water retention (36.4%). Improved water wettability of the pretreated fabrics is accompanied by increased water retention capacity, especially in the samples treated at neutral pH (pH 7 and P samples in Table II). The WRV of the fabric pretreated in acidic buffer without enzyme remains almost unchanged (35.0%). Adding enzymes results in a higher WRV. Celluclast 1.5 L increases the WRV of the substrate to a greater extent than Viscozyme 120L. Pretreatment in phosphate buffer with (P) or without enzyme (pH 7) improves the WRV to as high a level as alkaline scouring (41.1%).

Among the enzymes used in this study, only Celluclast 1.5 L causes a small weight loss (0.8%, see Table II). Neither pectinase nor xylanase results in measurable weight losses. However, scouring with sodium hydroxide causes a significantly greater weight loss (5.2%).

Color evenness of the desized fabric can be characterized by $\Delta E_{ab}^*$ = 0.1, and that value for conventionally scoured cotton fabric is 0.2 (Table II). Treatments with or without enzymes do not influence the color evenness of fabrics and do not cause inhomogeneities in the substrates. Most of the calculated $\Delta E_{ab}^*$ values are 0.2, so the human eye cannot detect color differences in the pretreated fabric samples.

Conventional scouring results in an extremely high color difference (8.4) with desized fabric (Table II, data based on desized fabric). An analysis of the constituents of $\Delta E_{ab}^*$ reveals that alkaline treatment increases the lightness (positive value for $\Delta L^*$) and decreases the color content (negative value for $\Delta C_{ab}^*$) significantly. Color difference values of the enzyme or buffer treated samples with desized fabric, however, are significantly lower (0.4–1.2), indicating less effective removal of natural coloring matter from cotton fibers. There is only a slight increase in lightness and a decrease in chroma during the processes. Changes in hue are negligible (i.e., $\Delta H_{ab}^*$ values near zero). Results show that buffer treatments have a nearly comparable cleaning effect, expressed as color change.

Comparing the color of enzyme and buffer treated fabrics with that of the alkaline scoured fabric (Figure 1, ▲), we see dramatic color differences, and the average value for $\Delta E_{ab}^*$ is 7.7. The extent of the differences in lightness and chroma is almost the same, but differences in hue are negligible (Table II, data based on conventionally scoured fabric). Results show that conventionally scoured fabric is significantly lighter and less colored than all of the enzyme and buffer treated samples. Color measurement proves and visual observation confirms that the color differences that exist between the conventionally scoured and bioscoured or buffer-treated fabrics are considerable. From the data in Table II, we see that while there is no difference in wetting properties between the bio- and conventionally scoured cotton fabrics, caustic scouring produces a significantly lighter and less colored fabric.

**Figure 1.** Color difference between biopretreated and alkaline scoured cotton fabrics after pretreatment (▲) and subsequent hydrogen peroxide bleaching (■). Pretreatments: pH 5 and pH 7 = buffer with surfactant, C = Celluclast 1.5 L, V = Viscozyme 120 L, P = Pulpyzme HC.

**Effect of Bleaching on Fabric Characteristics**

We examined the effects of hydrogen peroxide bleaching applied after the different pretreatments (enzymatic, buffer, caustic scouring) on the fabric properties. Data in Table III show that bleaching does not cause significant changes in the WRV of the samples pretreated with sodium hydroxide (CS) or xylanase enzyme (P). The most considerable differences in WRV that hydrogen peroxide produces are on those pretreated with acidic buffer and pectinase (+3.7 and +4.6%, respectively).

Color evenness data for the bleached fabrics ($\Delta E_{ab}^*$) prove that treatment with hydrogen peroxide solution does not cause divergences and perceptible color differences in the samples (Table III). The results also show
that the bleachability of the biopretreated fabrics is significantly better than that of the conventionally scoured sample. While bleaching of the conventionally scoured fabric results in a color difference of 4.6, that of the enzyme and buffer treated samples yields much higher changes in color: $\Delta E_{ab}^*$ values range from 10.2 to 12.1. This is revealed mainly by the higher positive lightness differences as well as the higher negative chroma differences with the appropriate pretreated samples.

After bleaching, all of the fabric samples (i.e., bioscourcd, buffer treated and alkaline scoured) had lightness values higher than 95 (data not shown), and the difference in lightness disappeared with hydrogen peroxide bleaching. Thus, owing to the bleaching, the lightness of the alkaline scoured fabric does not differ from that of the bioscourcd and buffer treated fabrics (Table III). This means that hydrogen peroxide bleaching after the pretreatment overcomes lightness differences that exist between the alkaline and biopretreated samples after pretreatment (see Table II). Data in Table III also show that while the differences in lightness disappear ($\Delta L^* \approx 0$), measurable chroma differences exist between the conventionally scoured and pretreated (enzyme or buffer) samples ($\Delta C_{ab}^* \approx 2$). Apparently, the bleaching step does not make the yellowish shade of the enzyme and buffer treated fabrics disappear, but only decrease. After bleaching, the bioscourcd and buffer treated fabrics still have a yellowish appearance, but their lightness and hue are almost the same as those of the alkaline scoured and bleached fabric.

Comparing the color differences between pretreated (enzyme and buffer) and conventionally scoured fabrics, measured after pretreatment (\(\Delta\)) as well as after hydrogen peroxide bleaching (\(\Delta\), Figure 1), it becomes obvious that hydrogen peroxide bleaching significantly reduces the color differences between the alkaline and biopretreated samples. Owing to the bleaching process, the average $\Delta E_{ab}^*$ changes from 7.7 to 2.1. An analysis of the constituents of the $\Delta E_{ab}^*$ reveals that while the pretreated (enzyme and buffer) samples are considerably darker, more colored, and yellower than the conventionally scoured fabric (Table II), after bleaching the untreated samples differ from the conventionally scoured samples only in chroma (Table III). We can summarize that application of a hydrogen peroxide bleaching step subsequent to biopretreatment is beneficial because it reduces the large color differences that exist between the conventionally scoured and biopretreated cotton fabric samples.

**Dyeing Behavior of Biopretreated Fabrics**

**Color Evenness After Reactive Dyeing**

Preparation may often be responsible for difficulties that only become apparent after coloration. Insufficient preparation can cause uneven dyeing, so careful pretreatment before dyeing is very important. It should ensure that the substrate has a high and uniform dye uptake and absorbency [8]. Although our results prove that absorbency and color evenness of the fabrics pretreated with enzyme or buffer are good enough and equal to those of the conventionally scoured fabric (see Tables II and III), it is worthwhile to characterize the dyeing behavior of the biopretreated fabrics, and to compare their color with that of the alkaline scoured and dyed fabric in detail. Elucidation of the effect of the subsequent bleaching step on the dyeing behavior of the biopretreated fabrics is also very important.

We first dyed the bioscourcd, buffer treated, and caustic scoured cotton fabric samples without bleaching, using a reactive dye at four concentrations in a bath exhaustion procedure. At a certain dye concentration, all six samples were dyed together in the same dyebath. $\Delta E_{ab}^*$ values calculated from the tristimulus values of ten readings from different positions on the swatch, indicating color evenness, are shown in Table IV ($\Delta E_{ab}^*$ without bleaching). All twenty-four dyed
samples appear homogeneous in color and free from any defects related to the dyeing process. Most of the $\Delta E_{ab}^* $ values are lower than 0.5, which is considered to be a visually unnoticeable color difference. Samples dyed at the highest dye concentration (2%) are slightly more even ($\Delta E_{ab}^* = 0.2$) than those dyed with lower, i.e., 1, 0.5, and 0.2% dye concentrations (maximum $\Delta E_{ab}^* $ of 0.3, 0.4, and 0.5, respectively). We can therefore summarize that biopretreatment results in homogeneously scoured fabrics that can be dyed uniformly. Thus, enzymes have no negative effect on color evenness of fabrics dyed with a reactive dye directly after biopretreatment. Visual perception agrees with the measured results, so biopretreatment does not cause perceptible color divergences in the cotton fabric samples dyed subsequent to enzymatic treatment with a reactive dye and without bleaching. Dyed samples pretreated in a buffer without enzyme also have a homogeneous appearance, and their evenness is no poorer than those of the bio- or conventionally pretreated and dyed samples. This means that enzyme and buffer treated samples are ready for subsequent dying without further oxidative bleaching.

We elucidate the effect of bleaching applied after the biopretreatment on the dyeing behavior of the fabric samples in the next experiment. Pretreated cotton fabric samples were first bleached with a hydrogen peroxide solution, then dyed. From the color evenness data in Table IV ($\Delta E_{ab}^* $ with bleaching), we see that hydrogen peroxide bleaching has no negative effect on the color evenness measured after dying. Most of the $\Delta E_{ab}^* $ values are lower than 0.4. There is no difference in the color evenness of enzyme and buffer treated samples; their evenness is excellent and equal to that of the alkaline scoured, bleached, and dyed fabric.

### Color Difference Between Conventionally Scoured and Biopretreated Samples After Reactive Dyeing: Effect of Chemical Bleaching After Pretreatment

Results in the previous sections show that great color differences exist between the biopretreated and conventionally scoured cotton fabric samples (Table II, Figure 1). Enzyme and buffer treated samples are significantly darker and more colored than the alkaline scoured fabric, and they have a yellowish appearance as well. Hydrogen peroxide bleaching subsequent to the pretreatments overcomes these differences in lightness and reduces the chroma differences significantly (Table III).

Dyeing of the fabric samples from the two experiments (i.e., pretreatment without or with bleaching) results in deviations. Figures 2a and b illustrate the color differences at 0.2 and 2% dye concentrations, respectively. (Results at 0.5 and 1% dye concentrations are not shown.) It is obvious from the data that without bleaching (▲), the color differences that exist between the alkaline scoured and biopretreated samples after dyeing are significantly greater than those for the dyed fabrics, which are bleached after the pretreatment and only subsequently dyed (■). Data also show that color differences depend largely on dye concentration. Without bleaching (▲), the average color differences are 4.4 and 2.6 at dye concentrations of 0.2 and 2%, respectively. With bleaching (■), the average color difference decreases from 1.2 to 0.6 with increasing dye concentration from 0.2 to 2%. The higher the dye concentration, the lower the color difference between the alkaline scoured and biopretreated samples after dyeing.

Visual evaluation of the dyed fabrics shows that without bleaching, the color differences at the dye concentrations of 0.2 and 0.5% are perceptible and too great to be acceptable. However, at higher dye concentrations (i.e., 1 and 2%), despite the measured higher color dif-
Bleachability and Dyeing Properties of Biopretreated and Conventionally

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FIGURE 2. Color difference between biopretreated and alkaline scoured cotton fabrics after reactive dyeing. Effect of dye concentration: (a) 0.2%, (b) 2%. Bleaching application: pretreatment and dyeing (▲), pretreatment, bleaching, and dyeing (■). Pretreatments: pH 5 and pH 7 = buffer with surfactant, C = Celluclast 1.5 L. V = Viscozyme 120 L, P = Pulzyme HC.

ference values, the bioscourcd and buffer treated samples conform to the conventionally scoured and dyed sample. Hydrogen peroxide bleaching between pretreatment and dyeing, at all the dye concentrations investigated, yields color differences that are not perceptible, and the pretreated (enzyme and buffer) samples match the alkaline scoured sample.

Lightness of the samples before dyeing affects the pale and medium dyeings significantly. Differences in color and mainly in lightness that exist before dyeing between the samples pretreated in different ways determine the color differences measured after dyeing at lower dye concentrations. Bleaching subsequent to the pretreatments decreases the chroma difference and overcomes the differences in lightness; thus, there is no considerable and visually observable color difference in the dyed samples pretreated by different methods.

Wash Fastness Properties

Even a perfect dyeing of a biopretreated sample may be rendered worthless if the fastness properties of the fabric are poor. For this reason, we have studied the wash fastness of the dyed samples. We assessed staining of the adjacent materials, i.e., cotton and wool, and color changes in the dyed samples that occur in wash fastness tests by means of the appropriate five-step gray scales, respectively. The results show that wash fastness values of the dyed fabrics do not differ and are optimal (grade 5) both for staining and color change. Wash fastness tests cannot determine differences between samples pretreated in different ways, or those bleached prior to dyeing.

Conclusions

We have used commercial cellulase, hemicellulasepectinase, and cellulase-free xylanase enzymes for biopreparation of desized cotton fabric. We have chosen a heterobifunctional reactive dye in four different concentrations to test dyeability and dyeing evenness of the pretreated samples. We have also investigated the effect of hydrogen peroxide bleaching applied subsequent to the enzymatic pretreatment on dyeing properties of the fabrics in detail. We compare the results for the biopretreated fabrics with those of fabric pretreated by a conventional alkaline method.

Enzymatic and buffer treatments in the presence of a nonionic surfactant yield fabrics with excellent and uniform water absorbency. Wetting time of the samples is equal to that of fabric scoured conventionally. When buffer is used with a surfactant without any enzymes, the wetting ability and absorbency of the desized cotton fabric also improve considerably. Treatments with or without enzymes do not influence the color evenness of fabrics and do not cause any inhomogeneity in the substrates. Conventionally scoured fabric appears much cleaner (less colored) and lighter than the bioscourcd and buffer treated samples, so there are great color differences between the biopretreated and alkaline scoured fabrics. However, a hydrogen peroxide bleaching subsequent to biopretreatment decreases these color differences significantly. Results prove that bleaching overcomes the lightness difference and reduces the chroma differences considerably. Bleachability of the enzyme and buffer treated fabrics is better than that of the conventionally scoured sample.

Biopretreated fabrics can be dyed subsequent to the enzymatic treatment without further oxidative bleaching. Reactive dyeing results in homogeneously dyed fabrics with a color evenness equal to alkaline scoured and dyed fabric. However, at lower dye concentrations (i.e., 0.2 and 0.5%), great color differences exist between conventionally scoured and biopretreated samples. At higher dye concentrations (i.e., 1 and 2%), on the other hand, there is no perceptible color difference between biopretreated and conventionally scoured samples. Thus, hydrogen peroxide bleaching after the pretreatments yields fabrics that can be dyed homogeneously with reactive dyes, and these dyed samples match the alkaline scoured fabric even if the dye concentration is low. Wash fastness of the dyed fabrics is excellent and seems to be independent of the pretreatment. There is essentially no difference in the dyeing properties of cotton fabrics subjected
to the buffer treatment alone and to the enzyme solution prepared in the same buffer.

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Role of EDTA Chelating Agent in Bioscouring of Cotton

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ABSTRACT

Our previous work demonstrated that ethylenediamine-tetra-acetic acid (EDTA) markedly enhances the apparent activity of commercial xylanase and pectinase enzymes in cotton bioscouring, accelerating degradation and removal of impurities. In this study, we explore how the EDTA can improve the efficiency of the enzyme process in cotton bioscouring. Effects of EDTA on the enzyme action, as well as on the degradation of seed-coat fragments – the most resistant natural impurities of cotton to be degraded in bioscouring - are investigated. Results show that application of EDTA in different concentrations does not inhibit the main activities of the enzymes investigated, thus pectinase activity of Viscozyme 120L, and xylanase activity of Pulpzyme HC retain almost unchanged. On the other hand, EDTA modifies the substrate structure by removing the calcium ions from the cross-bridges that link the macromolecules in pectin to one another or pectin to other polysaccharides. While EDTA pretreatment decreases significantly the efficiency of enzymatic hydrolysis monitored by reducing sugar liberation, simultaneous application of EDTA and enzyme in one treating bath accelerates the degree of hydrolysis very much indicating a synergistic effect of enzyme and EDTA.

Recent results in textile biotechnology proves that enzymes can be used effectively in the preparatory processes of natural cellulosic fibers like cotton and flax. In bioscouring of cotton the removal of the noncellulosic ‘impurities’ (i.e. waxes, pectic substances, proteins, lignin-containing and coloring materials, etc) can be achieved mainly by hydrolytic enzymes, such as pectinases, xylanases and cellulases. Enzymatic degradation of pectin accelerates the removal of waxy materials from the cotton primary wall, thus produces water wettable cotton. The bioprocess has several advan-
tages over traditional chemical scouring. Enzymes operate under mild conditions (pH, temperature) with low water consumption and act only on specific substrates. On the other hand, traditional alkaline scouring carried out with hot caustic soda is unquestionably an energy-, water- and chemical-intensive process [17].

Enzymes can also be used with good results in the retting process of flax. Attempts were made to eliminate the disadvantages of the current commercial method of field retting and to improve the quality and consistency of the fibers. As a result of the research focused on the development of alternative retting methods a specific enzyme-retting has been worked out. In the process pectinase-rich enzyme mixtures are used to separate the fibers from non-fiber tissues and to produce quality fibres from flax. Enzyme-retting is more controllable and environmentally-friendly than the conventional dew-retting process [1, 20].

Both in bioscouring of cotton and enzyme-retting of flax, degradation of pectic substances is the most essential process. Pectin generally builds up from D-galacturonic acid residues, which are present in a linear backbone. Some of the carboxylic acid groups are esterified with methanol. The pectic polymer can be called a block copolymer with alternating methyl-esterified and non-esterified blocks. The pectin is covalently linked to hemicellulose or to cellulose, or there are strong hydrogen-bonds between pectin and other components in the cell wall. On the other hand, pectin interacts itself forming calcium cross bridges, which link the pectin components to one another. The crosslinks are formed between the non-esterified or little methyl-esterified galacturonan blocks with negative charge and calcium ion with positive charge by electrostatic interactions. The calcium ion can bind pectin and other polysaccharides too. It generates coordination linkages with hydroxyl group of polysaccharides and ionic linkages with the carboxylic group of pectin [12, 10, 8]. An “egg-box” model was proposed to show how calcium ions strengthen plant cell walls [11]. Consequently, removal of calcium ions can accelerate the degradation of calcium-rich pectic substances.

Application of chelating substances to desiccated flax to enhance microbial-retting was first reported several years ago [19]. Comprehensive research works on enzyme retting of flax have shown that among several chelators tested, ethylenediaminetetraacetic acid (EDTA) is the most efficient chelator in improving enzyme retting of flax. Recently we have shown that addition of EDTA markedly enhances the apparent enzyme activity in cotton bioscouring [5]. EDTA improves the effectiveness of commercial xylanase and pectinase enzymes, as well as accelerates both the removal of the impurities from cotton fabric and the degradation of seed-coat fragments separated from the fabric. Application of EDTA in the enzyme solution has also significant effect on the efficiency of the subsequent chemical process.

In the present paper we explore how the EDTA can improve the efficiency of the enzyme process in cotton bioscouring. The effects of EDTA on the apparent activities of commercial pectinase and xylanase enzymes, and on the degradation of non-cellulosic constituents in bioscouring are investigated in detail. The main goals of this study were the quantitative characterization of enzyme activities in the presence of EDTA and comparison of the degradation efficiency of the non-cellulosic constituents of cotton in bioscouring with or without EDTA. Influence of EDTA in different concentrations on enzyme activities i.e. filter paper (cellulase), 1,4-β-endoglucanase, β-glucosidase, xylanase and pectinase activities, is investigated in detail. Degradation of seed-coat fragments – the most resistant impurities of greige cotton - is characterized in cotton bioscouring by weight loss and reducing sugar liberation, as well as by changes in calcium ion content.
Experimental

Viscozyme 120L (Aspergillus sp. origin), a pectinase-rich enzyme and Pulpzyme HC (Bacillus sp. origin), a pure xylanase were kindly supplied by Novozymes. The activities were measured according to the methods described in Refs. 9, 15, 3, 2, 22. Results are shown in Table I. The influence of EDTA on the activity of the enzymes was tested by performing the standard activity assay in the presence of EDTA, at the EDTA-enzyme ratio of 1; 3; 5; 6; 8.6 (mM EDTA/ml enzyme). All activity values reported in this paper are mean values of triplicate experiments. (Table I)

Spinning blowroom waste, a combined waste produced during the mechanical steps of cotton purification was selected to model the seed-coat fragment impurity. It consists of stalks leaves and seed-coat fragments of cotton [7]. Before use, the substrate was washed in distilled water at room temperature for 30 minutes to extract the water-soluble constituents and air-dried. Prior to the treatments the sample was conditioned at 65% RH and 21°C for 24 hours.

Four sets of treatments have been applied: (a) EDTA-only, (b) enzyme-only, (c) EDTA pretreatment and subsequent enzyme treatment, without drying between the two steps and (d) enzyme and EDTA applied together in one bath. EDTA treatment of seed-coat fragment samples (a) was carried out in phosphate buffer (pH 7) at 50°C for 15 minutes in agitated system (Scourotester laboratory unit, 40 ± 2 rpm). The liquor ratio was 1:100 and EDTA concentration was 0.5 mM/g substrate. Enzyme treatment (b) was performed in agitated system at pH 5 (0.05 M acetate buffer) for Viscozyme 120 L, or at pH 7 (0.05 M phosphate buffer) for Pulpzyme HC. The liquor ratio was 1:100, enzyme concentrations were 2 and 4 ml/L respectively, treatment time was 1 hour, incubation temperature was 50°C, and the concentration of the nonionic surfactant (Felosan TAK-NO, CHT R. Beitlich GmbH, Germany) was 1 ml/L. After enzymatic treatment, the substrate was washed twice in hot distilled water for 10 seconds each to deactivate the enzyme and then air-dried for 24 hours. In the experiment (c) the EDTA pretreatment of seed-coat fragment samples was carried out as described above (step a). Subsequent to filtration the substrate was treated with enzyme according to the method described earlier (step b). When enzyme and EDTA were used together (d) 0.5 mM EDTA/g substrate was added to the enzyme solution. The treatment was carried out similarly as described above (step b).

Weight loss was determined by weighing the samples before and after the treatment following 24 hours of conditioning at 20°C and 65% RH. Release of reducing sugars during the enzymatic treatments was determined in the reaction liquor using the 3,5-dinitrosalicylic acid reagent [16]. Elemental analysis of the enzymes and calcium ion content of the substrate i.e. cotton seed-coat fragments, were determined by Induced Coupled Plasma Optical Emission Spectroscopy (ICP-OES) method as described earlier [5].

Table I. Activities of commercial enzymes used throughout the experiments. a

<table>
<thead>
<tr>
<th>Enzymes</th>
<th>Cellulase (FPA), FPU/ml</th>
<th>1,4-β-endo-glucanase, EGU/ml</th>
<th>β-glucosidase, IU/ml</th>
<th>Pectinase, IU/ml</th>
<th>Xylanase, IU/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Viscozyme 120L</td>
<td>2 (0.3)</td>
<td>8600 (80)</td>
<td>4 (0.2)</td>
<td>7900 (380)</td>
<td>110 (3)</td>
</tr>
<tr>
<td>Pulpzyme HC</td>
<td>0</td>
<td>50 (0.8)</td>
<td>0</td>
<td>0</td>
<td>13800 (550)</td>
</tr>
</tbody>
</table>

a Standard deviations in brackets.
Results and Discussion

INFLUENCE OF EDTA ON ENZYME ACTIVITY

Viscozyme 120L is a pectinase rich enzyme with significant 1,4-β-endoglucanase activity. The enzyme complex shows negligible xylanase, cellulase and β-glucosidase activities too. Pulpzyme HC is almost a pure xylanase enzyme with practically zero cellulase (FPA), β-glucosidase and pectinase activities under standard assay conditions; a very small amount of 1,4-β-endoglucanase activity can be detected (Table I). Influence of EDTA on the enzyme activities of the two enzymes was investigated in more detail. EDTA with concentrations of 0 - 8.6 mM/ml enzyme was added to the enzyme solution and the standard activity assays were performed.

For Viscozyme 120L, the measured enzyme activities as a function of EDTA concentration are presented in Figures 1-5. As shown in Figures 1 and 2, EDTA has no effect on cellulase (FPA) and β-glucosidase activities. On the other hand, 1,4-β-endoglucanase (Figure 3), xylanase (Figure 4) and pectinase (Figure 5) activities are slightly inhibited by EDTA. With an EDTA concentration of 1 mM/ml enzyme, 1,4-β-endoglucanase activity is reduced by approx. 9% (from 8600 to 7800 EGU/ml, Figure 3). By increasing the EDTA concentration from 1 to 8.6 mM/ml only a minor further negative effect on 1,4-β-endoglucanase activity occurred. For xylanase activity, by adding 1 and 3 mM EDTA to the enzyme solution the initial activity (107 IU/ml) has been reduced to 103 and 93 IU/ml (Figure 4), respectively. Further increase in EDTA concentration from 3 to 8.6 mM/ml does not result in any further xylanase activity loss. It can be stated from the data in Figure 5 that EDTA does not affect significantly the pectinase activity, although a slight decrease can be measured at the first two EDTA concentrations (i.e. 1 and 3 mM/ml). At 3 mM EDTA/ml enzyme concentration the initial pectinase activity (7900 UI/ml) is reduced only by 8.8%.

For Pulpzyme HC, EDTA does not inhibit the xylanase activity significantly (Figure 6). Although the data vary near the initial activity value with the EDTA concentration, but no trend can be observed. EDTA causes measurable decrease in 4-β-endoglucanase activity (Figure 7). It can be summarized from the results that EDTA does not inhibit significantly the main activities of the enzymes investigated, thus pectinase activity of Viscozyme 120L and xylanase activity of Pulpzyme HC retain almost unchanged in the presence of EDTA.

Table II. Analysis of Viscozyme 120 L and Pulpzyme HC enzymes by ICP-OES method.

<table>
<thead>
<tr>
<th>Constituents</th>
<th>Viscozyme 120L</th>
<th>Pulpzyme HC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Al</td>
<td>2.3</td>
<td>18.5</td>
</tr>
<tr>
<td>Ca</td>
<td>13.2</td>
<td>2290</td>
</tr>
<tr>
<td>Fe</td>
<td>11.6</td>
<td>7.6</td>
</tr>
<tr>
<td>K</td>
<td>2860</td>
<td>56</td>
</tr>
<tr>
<td>Mg</td>
<td>101</td>
<td>114</td>
</tr>
<tr>
<td>Na</td>
<td>39600</td>
<td>344</td>
</tr>
<tr>
<td>P</td>
<td>1160</td>
<td>1250</td>
</tr>
<tr>
<td>S</td>
<td>721</td>
<td>427</td>
</tr>
</tbody>
</table>
There are many so-called metallo-enzymes that contain metal ion in their structure. By removing the metal ion by chelating, tertiary structure of the enzyme can be destroyed. As a consequence, the activity may decrease or disappear. It is also demonstrated that several bacterial pectate lyases require calcium ion for enzyme activity and they are inhibited by EDTA, but the inhibition can be reduced by addition of an excess of calcium ion \([18, 13]\). EDTA can also inhibit the activity of exopolygalacturonase by 20-80\% depending on the reaction conditions. However, the enzyme activity is not re-

FIGURE 1. Effect of EDTA on filter paper activity of Viscozyme 120 L enzyme.

FIGURE 2. Effect of EDTA on β-glucosidase activity of Viscozyme 120 L enzyme.

FIGURE 3. Effect of EDTA on 1,4-β-endoglucanase activity of Viscozyme 120 L enzyme.

FIGURE 4. Effect of EDTA on xylanase activity of Viscozyme 120 L enzyme.

FIGURE 5. Effect of EDTA on pectinase activity of Viscozyme 120 L enzyme.
stored at all by adding different divalent ions, such as calcium [14].

Among xylanases only one was found to have a calcium binding domain. However, the activity of the enzyme was not significantly influenced by either the disruption of the metal binding domain, or EDTA treatment. It was also suggested that endoxylanase from *Aspergillus versicolor* requires metal ion for enzyme action, because in the presence of EDTA the activity decreased almost by 50%. [21, 4].

Analysis of the two enzymes used in this research by ICP-OES method show that they contain approximately equal amounts of phosphorus, and magnesium (Table II). For Viscozyme 120L, the amount of sodium and potassium ions, and for Pulpzyme HC, the amount of calcium ion is significant. Our results prove that there is no significant activity loss in the presence of EDTA if Viscozyme 120 L or Pulpzyme HC enzymes are used together with EDTA. Thus, these metal ions are not part of the enzyme structure, and EDTA does not change the tertiary structure of the enzymes by chelating. The origin of metal ions in the commercial enzymes may be attributed to the medium ingredients used in fermentation and to the additives during the downstream processing.

**EFFECT OF EDTA ON COTTON BIOSCOURING (SEED-COAT FRAGMENT DEGRADATION)**

Seed coat fragment is a portion of a cotton seed, usually black or dark brown in color, broken from mature or immature seed, and to which fibers and linters may be or may not be attached. We found in our earlier experiments [6, 7] that mechanically attached (vegetable origin) contaminants i.e. seed-coat fragments, pieces of stem and leaf trash, are the most resistant impurities of cotton. They are more troublesome in pre-treatment than the other non-cellulosic constituents of the cotton fibers. Although, the different mechanical cleaning steps remove majority of the seed-coat fragments, a certain amount of these impurities may adhere to the fibers and may be present even in the final fabric. They differ in chemical composition, color and morphology from cotton cellulose. In the traditional chemical processes their removal requires more concentrated chemical solutions and longer steaming periods. Their removal is the greatest obstacle to commercialization of biopreparation process. Whether either the traditional chemical or the enzymatic process is used for preparation of cotton fabrics, special attention

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Weight loss, %</th>
<th>Calcium ion, ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td>- (untreated)</td>
<td>-</td>
<td>34900</td>
</tr>
<tr>
<td>EDTA b</td>
<td>10.6 (0.7)</td>
<td>18500</td>
</tr>
<tr>
<td>Viscozyme 120L c</td>
<td>14.8 (0.5)</td>
<td>17700</td>
</tr>
<tr>
<td>EDTA + Viscozyme 120L d</td>
<td>22.5 (0.7)</td>
<td>13200</td>
</tr>
<tr>
<td>Viscozyme 120L with EDTA e</td>
<td>21.5 (0.6)</td>
<td>11900</td>
</tr>
<tr>
<td>Pulpzyme HC c</td>
<td>14.1 (0.6)</td>
<td>31100</td>
</tr>
<tr>
<td>EDTA + Pulpzyme HC d</td>
<td>25.0 (0.6)</td>
<td>16000</td>
</tr>
<tr>
<td>Pulpzyme HC with EDTA e</td>
<td>22.2 (0.6)</td>
<td>16800</td>
</tr>
</tbody>
</table>

a Standard deviations in brackets. b Treatment with EDTA. c Treatment with enzyme. d Pretreatment with EDTA and subsequent enzyme treatment. e Enzyme treatment with EDTA.
has to be paid to the degradation of seed-coat fragments.

Metal ion content of cotton seed-coat fragments is extremely high comparing to that of the cotton fiber itself [5]. According to ICP-OES determinations, for example the amount of calcium ions in loose cotton fiber is about 500 ppm, whereas the quantity of the same cation in the seed-coat fragments is as high as 35000 ppm (Table III). Pectin substances in plant usually form aggregates with calcium ions that crosslink pectin chains to each other and to other polymers of the lignin-holocellulose composite. Therefore, removal of calcium ions from the substrate with chelators may accelerate the enzyme action and promote the degradation of seed-coat fragments in biopreparation process.

Seed-coat fragment samples were subjected to a cold distilled water extraction first and then to different treatments, i.e. EDTA-only, enzyme-only, enzyme subsequent to the EDTA pretreatment and co-application of enzyme and EDTA in one bath. The overall degradation of the solid materi-

FIGURE 6. Effect of EDTA on xylanase activity of Pulpzyme HC enzyme.

FIGURE 7. Effect of EDTA on 1,4-β-endoglucanase activity of Pulpzyme HC enzyme.

FIGURE 8. Treatment of cotton seed-coat fragments by pectinase (a) and xylanase (b). The enzymes have been used directly to the substrate, or after EDTA pretreatment of the fragments, or together (in one bath) with EDTA.
als were characterized by the weight loss (Table III). EDTA treatment alone causes 10.6% loss in weight, and the initial amount of calcium ions has been reduced by 47% owing to the chelating process. Both pectinase and xylanase treatments result in higher weight losses at 14.8 and 14.1%, respectively. Note that while the weight loss values for the two enzyme treatments are essentially the same, the pectinase treatment in acidic buffer decreases the calcium content enormously from 34900 to 17700 ppm, while the xylanase treatment at pH 7 causes only a milder decrease (to 31100 ppm).

The two-step process, i.e. EDTA pretreatment and a subsequent enzyme treatment proves to be the most effective in degradation of seed-coat fragment samples, yielding 22.5 and 25.0% weigh loss values for Viscozyme 120L and Pulpzyme HC, respectively. Both the EDTA pretreatment and the subsequent acidic pectinase treatment (EDTA + Viscozyme 120L) contribute significantly to release the calcium from the substrate, leaving the residual calcium content of 13200 ppm. For EDTA + Pulpzyme HC two-step treatment, the effect of EDTA is more remarkable than the action of enzyme. For simultaneous treatment with enzyme and EDTA, the weight loss values are higher as compared to the single use of enzyme or EDTA, however these figures are less than those from the two-step EDTA + enzyme treatment.

While weight loss data characterized the overall degradation effect of the applied treatments, reducing sugars in the treatment filtrates reveal the effectiveness of the enzyme action. As Figures 8a and b show, the EDTA pretreatment decreases significantly the hydrolysis level in the subsequent enzyme treatment, indicating changes in substrate structure by extraction of the divalent ions. The amount of solubilized sugars decreases by 70 and 22% for pectinase and xylanase, respectively. Removal of the calcium cross bridges by EDTA pretreatment can cause a temporarily open structure, which can collapse by the end of the pre-treatment, resulting in a less accessible structure for the enzyme macromolecules applied subsequently.

On the other hand, addition of EDTA into the enzyme solution can accelerate the hydrolysis degree enormously (Figures 8a and b). Amount of reducing sugars increases by 72 and 38% in the bath of pectinase and xylanase enzymes, respectively, indicating synergistic effect of the enzymes and EDTA applied together in one treating bath. Removal of calcium ions from calcium cross-bridges in hemicelluloses by EDTA results in somewhat free and accessible areas in the substrate where the enzyme present is prompt to act in catalysis of chain-cutting process. Thus, in this case the enzymes are capable of accessing the macromolecules released in the simultaneous chelating process.

Taken altogether, EDTA has significant effect on the efficiency of the enzymes i.e. pectinase and xylanase, since in both pectin and xylan polymers there are divalent ion cross-bridges. Their removal modifies the polymer structure and alters its accessibility for the enzymes.

Conclusions

The main goal of this study was to examine how EDTA is effective in cotton bioscouring with commercial pectinase and xylanase enzymes. Influence of EDTA on the enzyme action and enzymatic degradation of the chosen substrate i.e. cotton seed-coat fragment was investigated in detail. Our findings show that EDTA added in different concentrations to the enzymes, does not have any inhibitory or stimulating effect on the main activities, i.e. pectinase and xylanase for Viscozyme 120L and Pulpzyme HC, respectively.

For enzymatic degradation of cotton seed-coat fragments, EDTA modifies the substrate structure by removing the calcium ions from the cross-bridges that link the macromolecules in pectin to one another or...
pectin to other polysaccharides. While EDTA pretreatment decreases significantly the efficiency of enzymatic hydrolysis monitored by reducing sugar liberation, simultaneous application of EDTA and enzyme in one treating bath accelerates the degree of hydrolysis very much. EDTA applied simultaneously with the enzyme, seems to assist in the creation of free and accessible areas for the enzyme present, indicating synergistic effect of the enzyme and EDTA. However, EDTA pretreatment results in a less accessible substrate for the enzyme applied subsequently, most likely due to the collapse of the temporarily open structure.

ACKNOWLEDGEMENTS

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Literature Cited


Influence of EDTA Complexing Agent on Biopreparation of Linen Fabric

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In this work the ‘EDTA–enzyme–substrate’ interaction was investigated in linen biopreparation. The effect of EDTA on the degradation of non-cellulosic constituents during bioscouring with pectinase enzyme was investigated in detail. Greige linen fabric was treated with pectinase, pectinase supplemented with ethylenediamine-tetra-acetic acid (EDTA), and EDTA pre-treatment followed by pectinase, in a tumble agitated bath. Adding EDTA to the enzyme solution accelerated the degree of hydrolysis and resulted in higher reducing sugar liberation and more efficient calcium ion extraction, indicating a synergistic effect of enzyme and EDTA. However, when EDTA was applied as a pre-treatment, a decrease in the efficiency of the subsequent enzymatic hydrolysis was observed.

Keywords: Linen; Enzyme; Biopreparation; Pectinase; EDTA; Calcium ions

INTRODUCTION

Biotechnology offers a wide range of alternative environmentally-friendly processes for the textile industry to complement or improve conventional technologies. Biopreparation of cellulose fibrils is an enzyme-aided process by which the noncellulosic ‘impurities’ (i.e. waxes, pectic substances, proteins, lignin-containing and coloring materials, etc.) are removed mainly by pectinase rich enzymes. Enzymes act under mild conditions (pH, temperature) with low water consumption; while the traditional alkaline scouring carried out with hot caustic soda is unquestionably an energy, water and chemical-intensive process. Thus, bioscouring provides an environmentally-friendly alternative to the conventional alkaline process (Nierstrasz and Warmoeskerken, 2003).

Numerous studies have been carried out on biopreparation of cotton (Buchert et al., 2000; Buschle-Diller et al., 1998; Etters, 1999; Lenting et al., 2002; Li and Hardin, 1998; Traore and Buschle-Diller, 2000; Tzanov et al., 2001). The results clearly show that, in bioscouring of cotton, degradation of pectic substances is one of the most essential processes. Pectin generally builds up from 1,2-galacturonic acid residues where some of the carboxylic acid groups are esterified with methanol. Pectin can be covalently linked to hemicellulose or to cellulose, or there can be strong hydrogen-bonds between pectin and other components in the cell wall. Pectin can also interact with itself forming calcium cross bridges between the non-esterified or partially methylated galacturonan blocks with negative charge, and the positively charged calcium ion. The calcium ions can also bind pectin and other polysaccharides. Therefore calcium ions as well as the interactions between calcium ions and cell walls play a key role in plant physiology (Demarty et al., 1984; Grant, 1997; Grant et al., 1973 Jarvis, 1984). Consequently, removal of calcium ions can accelerate the degradation of calcium-rich pectic substances.

Several researchers have reported that the joint effect of complexing agents and pectinase-rich enzymes in the bioprocessing of greige cellulose substrates can increase the efficiency of the cleaning process (Lenting et al., 2002; Takagishi et al., 2001; Durden et al., 2001). We have shown recently (Csiszár et al., 2001) that EDTA markedly enhances the apparent activity of commercial xylanases and pectinases in cotton bioscouring, accelerating the degradation and removal of impurities. It has also been demonstrated (Losonczy et al., in press) that application of EDTA in different concentrations neither inhibits or increases the activities of pectinase in a pectinase-rich enzyme complex, or xylanase activity in a pure xylanase preparation. However, the mode of EDTA application has a

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significant effect on the degradation of seed-coat fragments in cotton bioscouring. While EDTA, applied as a pretreatment significantly decreases the efficiency of the subsequent enzymatic hydrolysis, monitored by reducing sugar liberation, simultaneous application of EDTA and enzyme in one treatment, accelerates the degree of hydrolysis very much indicating a synergistic effect of enzyme and EDTA.

EDTA can also accelerate the microbial-retting of flax (Sharma, 1986; Henriksson et al., 1997). Comprehensive research work on enzymatic retting of flax has shown that among several chelators tested, EDTA is the most efficient in improving enzyme performance (Adamsen et al., 2002).

In this work a pectinase-rich commercial enzyme in combination with EDTA has been used for the biopreparation of linen. Greige linen fabric was subjected to three different treatments, i.e. pectinase-only, co-application of pectinase and EDTA in one bath, and a pectinase treatment subsequent to EDTA pretreatment. Linen contains only about 70% of cellulose and a high proportion of non-cellulosic matter, i.e. hemicelluloses, pectic substances, lignin, water-soluble and waxy materials. The influence of EDTA on the enzyme performance, as well as on the degradation of the non-cellulosic constituents during biopreparation was investigated in detail. The main goal of this study was the comparison of the degradation efficiency of the non-cellulosic constituents of linen in biopreparation with or without EDTA.

**MATERIALS AND METHODS**

100% greige linen fabric, plane-weave, supplied by Pannon-Flax Linen Weaving Co. Győr, Hungary, with a yarn count of 73/75 warp/weft and a fabric weight per unit area of 211 g/m² was used. The fabric samples (12 × 20 cm, approximately 5 g) were sewn around the edges to prevent unraveling during processing. They were conditioned at 65% relative humidity and 20°C for 24 hours prior to any treatment and testing. An acidic pectinase-hemicellulase (Viscozyme L, *Aspergillus* sp.), a gift from Novozymes A/S, 2880 Bagsvaerd, Denmark, was used for the biopreparation. The following enzyme activities were measured in the Viscozyme L using internationally recognized methods: Filter paper cellulase activity (Ghose, 1987): 3.0 (± 0.2) FPU/ml; β-Glucosidase activity (Kubicek, 1982): 65 (± 4) IU/ml; 1,4-β-Endoglucanase activity (Bailey and Nevalainen, 1981): 5100 (± 60) EGU/ml; Xylanase activity (Bailey et al., 1992): 150 (± 10) IU/ml; Pectinase activity (Wood and Kellogg, 1988): 5200 (± 340) IU/ml.

Three sets of treatments have been applied: (a) enzyme-only, (b) enzyme and EDTA applied together in one bath, (c) EDTA pretreatment and a subsequent enzyme treatment, without drying between the two steps. Enzyme treatment (a) was performed in Scourtestery laboratory unit (40 ± 2 rpm) with tumble agitation at pH 5 (0.05 M acetate buffer). The liquor ratio was 1:50, enzyme concentration was 1 ml/L, treatment time was 1 hour, incubation temperature was 50°C, and the nonionic surfactant concentration was 1 ml/L. The effect of different liquor ratios on the biopreparation of cellulose substrates in laboratory-scale experiments had been tested previously. For greige linen fabric, of the three liquor ratios tested, 1:50 produced the fabric with the best color evenness ($ΔE^*_{ab} < 0.5$).

After enzymatic treatment, the fabric was washed twice in hot distilled water (approx. 98°C) for 10 seconds each to deactivate the enzyme and then air-dried. When enzyme and EDTA were used together (b) 0.1 mM EDTA/g fabric was added to the enzyme solution. The treatment was carried out similarly to the method above (step a). In experiment (c) the EDTA pretreatment was carried out in acetate buffer (pH 5) at 50°C for 15 minutes in an agitated system (Scourtestery laboratory unit, 40 ± 2 rpm). The liquor ratio was 1:50 and the EDTA concentration was 0.1 mM/g fabric. After the EDTA pretreatment the fabric was passed through the laboratory paddle to reach an average wet pickup of 100 ± 5%, and then it was treated with enzyme according to the method described earlier (step a). All experiments were done in triplicate. For control treatments, buffer only, buffer and EDTA applied together in one bath, and EDTA pretreatment and a subsequent buffer treatment were performed.

**Fabric Evaluation**

Weight loss was determined by weighing the samples before and after treatment following 24 hours of conditioning at 20°C and 65% relative humidity. Release of reducing sugars during the enzymatic treatments was determined in the reaction liquor using the 3,5-dinitrosalicylic acid reagent (Miller, 1959) Calcium content of the fabrics was determined by Induced Coupled Plasma Optical Emission Spectroscopy (ICP-OES) method as described earlier (Csiszár et al., 2001).

Wettability of the fabrics was characterized by the water drop test, counting the elapsed time between the contact of the water drop with the fabric and the disappearance of the drop into the fabric. Ten readings were taken from different locations on the sample after treatment and the average was reported. The measurement was per-
formed on conditioned fabric and in a conditioned environment (at 20°C and 65% relative humidity).

Breaking load and elongation of the fabric at rupture were measured for both the warp and weft directions, by raveled strip test (ASTM 1682, test width and length of fabric strip, 2.5 x 10 cm) on an Instron Tester Model 5566.

Color tests were performed with a Hunterlab Color QUEST (D65/10°) colorimeter and evaluated according to the CIELab color space. Each sample measurement represents the average of five readings from different positions on the swatch. Efficiency of the processes was characterized by the color difference values, relative to the untreated greige fabric.

RESULTS AND DISCUSSION

Weight Loss, Reducing Sugar Liberation, Calcium Content

Greige linen fabric samples were subjected to different treatments, i.e. pectinase-only, co-application of pectinase and EDTA in one bath, pectinase after EDTA pretreatment, as well as the respective control treatments in agitated system for 1 hour. The overall degradative effect of the treatments is characterized by the fabric weight loss (Fig. 1). Treatment with acidic pectinase caused a small (2.3%) weight loss. Addition of EDTA to the enzyme solution did not cause a significantly higher weight loss (2.4%). The two-step process, i.e. EDTA pretreatment followed by enzyme treatment, proved to be the most effective in the removal of the noncellulosic impurities from greige linen, yielding a 3.5% weight loss. The results in Fig. 1 also show that weight loss data are significantly higher for the samples treated with pectinase, compared to the respective buffer treated controls.

![Graph 1](image1.png)  
**FIGURE 1** Weight loss of linen fabrics. Effect of EDTA on the enzyme action in biopreparation of linen fabrics.

![Graph 2](image2.png)  
**FIGURE 2** Effect of EDTA on reducing sugar liberation in the biopreparation of linen fabrics.

While weight loss data characterize the overall impurity-removal of the applied treatments, reducing sugars in the treatment filtrates exclusively reveal the effectiveness of the enzyme action in the processes. As Fig. 2 shows, addition of EDTA into the enzyme solution can accelerate the hydrolysis degree significantly. The amount of reducing sugars increased by 13%, indicating a synergistic effect of enzyme and EDTA applied together. On the other hand, EDTA pretreatment reduced the level of hydrolysis in the subsequent enzyme treatment, decreasing the reducing sugars by 17%. For all controls, treatment of greige linen fabrics in the respective buffer solutions did not result in reducing sugar liberation.

Pectic substances in plants usually form aggregates with calcium ions that crosslink pectin chains to each other. Therefore, the decrease of the calcium ions may promote the degradation of pectin. According to ICP-OES determination, the calcium ion content of the greige linen fabric was about 2070 ppm (Fig. 3). Treatment with the acidic pectinase reduced the initial amount of the calcium by 40% to 1250 ppm. Co-application of the enzyme and EDTA decreased the calcium content by 80%, leaving a residual calcium content of 416 ppm. However, the two-step process, i.e. EDTA pretreatment and subsequent enzyme treatment was less effective in the removal of calcium than the pectinase with EDTA treatment, resulting in a 67% decrease. For control treatments without enzyme, the mild acidic medium (pH 5, acetate buffer) is very efficient in removing the calcium ions from the substrate. Buffer only and buffer with EDTA treatments release about 30% and 73% of the calcium ions, respectively. Both are less effective than the respective enzyme treatments. The two control treatments including EDTA, i.e. buffer with EDTA, and EDTA pretreatment and a subsequent buffer treatment, cause almost the same
Appendix IV.

FIGURE 3  Effect of EDTA on the residual calcium ion content of the biopretreated linen fabrics.

decrease in calcium content of the linen fabric (73 and 75%, respectively).

Thus, the synergistic effect of the enzyme and EDTA applied together results in higher reducing sugar liberation and more efficient calcium ion extraction. From the results it seems that removing the calcium ions from calcium cross-bridges in pectin with EDTA, in a simultaneous chelating process, yields accessible areas in the substrate structure where the enzyme can catalyse the chain-cutting process. However, in the two-step process, i.e. an EDTA pre-treatment and a pectinase treatment thereafter, it is most likely that the partially accessible substrate structure collapses by the end of the pretreatment, resulting in a more unsuitable structure for the enzyme macromolecules applied subsequently. This is revealed mainly by the lower amount of the solubilized sugars, as well as the less effective calcium ion extraction.

Breaking Load and Elongation, Wettability, Changes in Color

A comparison of the breaking load and elongation, determined after the biopreparation processes without or with EDTA, can be made from the data in Table I. Results prove that none of the applied biopreparation systems has any drastic damaging effect on the linen fabric. Data show that the acidic pectinase-rich enzyme treatmentweakens the warp yarns only by 5% and does not alter the breaking load of linen fabric in the weft direction at all. Adding EDTA into the enzyme solution causes a slightly higher decrease in the warp breaking load (16%), but does not change the value of the weft strip significantly. For the two-step process, i.e. EDTA pretreatment and the subsequent enzyme treatment, the loss in warp breaking load is equal to that of the pectinase-only treated fabric (16%), thus applying EDTA as a pretreatment does not modify the degrading effect of the subsequent enzyme treatment for the warp yarns. However, among the biopreparation systems used in this study, only the two-step process causes a slight degradation in the weft yarns, resulting in a 12% decrease in the weft-breaking load. While the pectinase–EDTA systems applied in the biopreparation have only a negligible effect on the resilience of the linen fabrics (Table I), the control treatments cause significant loss in breaking elongation of the linen fabrics.

Wetability of the linen fabrics was characterized by the water drop test. Table II presents the wetting time data obtained for all enzyme–EDTA combinations and control treatments. All six fabrics have a wetting time of near or less than 1 second. Therefore, the water drop test does not reveal significant differences between the treatments. Averaged values of wetting time calculated from ten determinations from varying locations on the linen fabric subsequent to the pretreatments show that none of the readings on randomly selected locations indicated an inhomogeneous fabric surface and non-uniform absorbency.

For color change, all pectinase biotreatments with or without EDTA result in a high and visually observable color difference compared to the untreated greige linen fabric (Fig. 4). The color difference value was 2.6 for the fabric treated with the acidic pectinase enzyme. Addition of EDTA to the enzyme solution caused only a slight increase in color difference (2.8). The two-step process, i.e.

<table>
<thead>
<tr>
<th>Biopreparation system</th>
<th>Breaking load (N)</th>
<th>Breaking elongation (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Warp</td>
<td>Weft</td>
</tr>
<tr>
<td>Untreated</td>
<td>362  (32)</td>
<td>475  (33)</td>
</tr>
<tr>
<td>Buffer</td>
<td>342  (5)</td>
<td>440  (28)</td>
</tr>
<tr>
<td>Pectinase</td>
<td>341  (37)</td>
<td>494  (59)</td>
</tr>
<tr>
<td>Buffer with EDTA</td>
<td>380  (9)</td>
<td>448  (31)</td>
</tr>
<tr>
<td>Pectinase with EDTA</td>
<td>302  (20)</td>
<td>481  (27)</td>
</tr>
<tr>
<td>EDTA pretreatment + Buffer</td>
<td>365  (7)</td>
<td>417  (38)</td>
</tr>
<tr>
<td>EDTA pretreatment + Pectinase</td>
<td>341  (29)</td>
<td>418  (18)</td>
</tr>
</tbody>
</table>
EDTA pretreatment and the subsequent enzyme treatment, resulted in the highest color change, with the average value for DE* being 3.6. Data also showed that color difference values of the control samples with greige fabric are significantly lower, indicating less effective removal of natural coloring matter from linen fibers. Analysis of the constituents of DE* reveals (Table III) that all treatments increase the lightness (positive value for DL*) and decreases the color content (negative value for DC*) significantly. Changes in hue (DH*) are negligible. The two-step process proved to be the most effective in the removal of coloring matter from greige linen. Comparing the color constituents of the pectinase treated fabric with that of the pectinase with EDTA treated fabric it can be seen that adding EDTA into the enzyme solution increases the lightness, resulting in higher DL* value. Chroma difference (DC*) value of the pectinase with EDTA treated fabric, however, is slightly lower, than that of the pectinase-only treated sample.

CONCLUSIONS

The main goal of this study was to compare the degradation efficiency of the non-cellulosic constituents of linen in pectinase-aided biopreparation with or without EDTA in a tumble agitated bath. Treated samples were characterized by weight loss, reducing sugar liberation, calcium ion content, breaking load and elongation, wetability and changes in color. Results show that adding EDTA into the enzyme solution accelerates the degree of hydrolysis, yielding higher reducing sugar liberation that is accompanied by increased calcium ion extraction. However, EDTA applied only as a pre-treatment, decreased the efficiency of the subsequent enzymatic hydrolysis.

None of the applied biopreparation systems has any drastic damaging effect on the linen fabric or modifies the fabric resilience. All three biotreatments—accomplished with or without EDTA—yield fabrics with good and uniform water absorbency and result in high and visually observable color differences from the untreated greige linen fabric.

Acknowledgements

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References

Appendix IV


