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Research Article

Wax removal for accelerated cotton scouring with alkaline pectinase

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A rational approach has been applied to design a new environmentally acceptable and industrially viable enzymatic scouring process. Owing to the substrate specificity, the selection of enzymes depends on the structure and composition of the substrate, *i.e.* cotton fibre. The structure and composition of the outer layers of cotton fibre has been established on the basis of thorough literature study, which identifies wax and pectin removal to be the key steps for successful scouring process. Three main issues are discussed here, *i.e.* benchmarking of the existing alkaline scouring process, an evaluation of several selected acidic and alkaline pectinases for scouring, and the effect of wax removal treatment on pectinase performance. It has been found that the pectinolytic capability of alkaline pectinases on cotton pectin is nearly 75% higher than that of acidic pectinases. It is concluded that an efficient wax removal prior to pectinase treatment indeed results in improved performance in terms of hydrophilicity and pectin removal. To evaluate the hydrophilicity, the structural contact angle (θ) was measured using an auto-porosimeter.

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1 Introduction

1.1 General

Scouring of cotton textiles is an essential treatment in wet textile processing to obtain a sufficiently hydrophilic fabric [1–4]. In cotton, non-cellulosic materials create a physical hydrophobic barrier to protect the fibre from the environment throughout its development. During scouring, waxes and other hydrophobic materials are removed from the cotton fibres. In aqueous textile processing the waxes and pectins impede wetting and wicking, subsequently obstructing aqueous treatments [5]. Conventionally, scouring is done in a hot aqueous solution of NaOH to remove hydrophobic components from the primary wall, *e.g.* pectin, protein and organic acids and the cuticle–waxes and fats [1, 3, 4]. However, alkaline scouring is a non-specific process. The use of high concentrations of NaOH also requires neutralization of wastewater. Even though alkaline scouring is effective and the costs of NaOH are low, the scouring process is rather inefficient because it consumes large quantities of water and energy. It is clear that this process needs to be improved considerably to meet today's energy and environmental demands. In the last couple of years, substantial research has been directed towards replacing this process with an enzymatic one [4–23].

Most of the research done to develop an innovative enzymatic scouring process was focused on the potentials of different enzymes. Cellulases and pectinases were identified as potential candidates for an enzymatic scour-

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Abbreviations: θ, structural contact angle; **PG**, polygalacturonases; **PL**, pectate lyase; **PVD**, pore volume distribution

ing process [6–10, 16, 24]. However, the application of pectinases resulted only in modest improvements in fabric wettability, and cellulases caused significant losses in fibre strength [10].

Therefore, a systematic approach has been applied here to understand and to design a new bioscouring process. Owing to the high substrate specificity of most enzymes, it is necessary to have sufficiently detailed information about the substrate composition and morphology to develop and introduce an economically viable, environmentally friendly bioscouring process. A thorough literature review has been done on this issue, including the latest insights from plant science. That reveals structural information that directly and strongly influences the selection of enzymes for developing a new scouring process. The gathered information about the cotton fibre is focused on those aspects that can affect the performance of an enzymatic scouring process.

Three main issues are discussed here: Firstly, a benchmarking of the existing alkaline scouring process, done by measuring the hydrophilicity after scouring in terms of the structural contact angle (θ) and the percentage pectin removal; secondly, an evaluation for several selected acidic and alkaline pectinases for cotton scouring; and finally, the proof for our hypothesis, which states that wax removal, prior to pectinase treatment, improves the enzyme performance. In this way pectinase can be used efficiently in the scouring process. *n*-Hexane, a non-polar solvent, was used to remove the wax from cotton to measure the full potential of the chosen pectinase. It must be noted here that the purpose of the *n*-hexane treatment was to prove the significance of a wax removal step prior to the enzymatic treatment and not to introduce *n*-hexane as a pre-treatment agent.

1.2 Cotton fibre structure and proposed hypothesis

Comprehensive information about the cotton fibre structure has been gathered from literature [2, 3, 25–34]. The total amount of non-cellulosic materials is dependent on the source of cotton and the maturity of the fibre. The outermost layer is the cuticle; it is a thin film mostly consisting of fats and waxes (0.4–1.2% of the cotton weight) and causes the hydrophobicity of the fibres [4, 6, 32]. Based on electron microscope examination, the cotton fibre cuticle has been shown to be very thin with a thickness of approximately 12 nm [25, 26, 28]. Table 1 gives a detailed description of the composition of the waxy material, having a rather high and wide range of melting points (64–214°C). The waxy contents can be divided into two categories, a saponifiable part (nearly 40% of total wax content) and a non-saponifiable part, comprising around 60% of the total wax. Alcohols such as gossypol ($C_{30}H_{30}O_8$), montanyl ($C_{28}H_{57}OH$) and ceryl ($C_{28}H_{53}OH$) are high molecular weight monohydric alcohols and belong to the category of non-saponifiable waxes [27]. These *n*-primary alcohols (C_{26}–C_{36}) combined with the fatty acids (C_{16}–C_{36}) are the main components of wax from the mature white cotton fibre. After treatment with boiling NaOH, waxes are hydrolysed into a sodium salt of the fatty acid and alcohol [2, 35].

Apart from the above-mentioned components in the cuticle, there are also some complex biopolymers present. Cutin, one of the biopolymers, is high molecular weight polyester that comprises various inter-esterified C_{16} and C_{18} hydroxy and hydroxy-epoxy fatty acids [26, 34]. The cutin biopolymer in the cuticle is formed by cross-linking hydroxylated fatty acids by intermolecular ester bonds, leading to a 3D structure [37, 38]. Luque *et al.* [39] proved, on the basis of Fourier-transform infrared spectroscopical analysis and X-ray diffraction analysis, that cutin has an amorphous structure. A study on the waxy composition of white cotton fibre supports the presence of cutin in the cuticle of cotton fibre. Such a composition is typical for waxes that are embedded in plant cuticular membranes, the so-called intra-cuticular waxes.

To destabilise the primary wall with enzymes, it is essential to study the interconnections of cellulosics and non-cellulosics. As illustrated in Figure 1, cellulose microfibrils are embedded in and linked to a matrix that contains hemicellulose, acidic pectins, esterified pectins and fibrous glycoproteins [30, 33, 40–42]. Hemicellulose molecules (*e.g.* xyloglucans) are linked by hydrogen bonds to the surface of the cellulose microfibrils. The backbone of hemicellulose is similar to that of cellulose while, xylose, galactose and fucose (the polysaccharides) are the additional residues present on the hemicellulose backbone. Some of these hemicellulose molecules are cross-linked to acidic pectin molecules (*e.g.* rhamnogalacturonan) through short and esterified pectin molecules (*e.g.* arabinogalactan). Rod-shaped glycoproteins are tightly woven into the matrix. These non-cellulosic components are present throughout the primary cell wall. When approaching the secondary cell wall, there is a difference in the distribution and nature of each component. In the secondary cell wall, crystalline cellulose is mainly present [3].

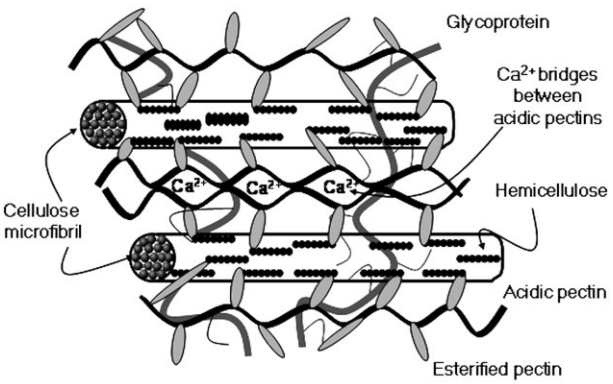

Figure 1. Interconnections between cellulose and other non-cellulosic components in the primary wall of the cotton fibre. Modified from [40].

Table 1. Detail wax composition of the mature dry cotton fibre [2, 3, 25, 27–29]

Type	Main Constituents	% dry weight	Details of each constituents				
			Constituent	Chemical formula	Molecular weight	Melting point (°C)	Remark
Unsaponifiable part 52–62%	High molecular weight alcohol C_{23}–C_{34}	40–52	*n*-Triacontanol	$C_{30}H_{61}OH$	438.8	87	Major
			Gossypol (colouring matter)	$C_{30}H_{30}O_8$	518.5	184–214	Major
			Montanyl (1-octacosanol)	$C_{28}H_{58}O$	410.8	83	Major
			Octacosanol	$C_{28}H_{58}O$	410.7	83	Traces
			Other alcohol	C_{28}-C_{30}	-	-	-
			Glycerol (low molecular weight alcohol, esterified with fatty acid)				
	Saturated and unsaturated hydrocarbons	7–13	Heptaconsane	$C_{27}H_{56}$			
			Triacontane	$C_{30}H_{60}$			
			Untriacontane	$C_{31}H_{64}$			
			Dotriacontane	$C_{32}H_{66}$			
	Phytosterol, sterol, glucoside and polyterpens	3–8	β-Sitosterol	$C_{20}H_{50}$	414.7	140	
			γ-Sitosterol	$C_{20}H_{50}$	414.7	147	
			Sitosterol glucoiside (sitosteroline)	$C_{35}H_{60}O_6$			
			α- and β-Amyrin	$C_{30}H_{50}O$			
Saponifiable part 37–47%	Fatty acid (with even number of carbon atoms) in free or esterified form	23–47	*n*-Tetracosanic acid (Lignoseric acid)	$C_{21}H_{43}O_{20}$	368.6	84	Major
			n-pentadecanoic acid	$C_{15}H_{30}O_2$	242.0	69.6	Traces
			Hexadecanoic acid (Palmatic)	$C_{16}H_{32}O_2$	256.4	64	Major
			cis-9-Octadecanoic acid (Oleic)	$C_{18}H_{34}O_2$	282.5	04	Traces
			Octadecanoic acid (Steric)	$C_{18}H_{36}O_2$	284.5	70	Major
			Iso-Behenic acid	$C_{22}H_{44}O_2$	340.6	80	Traces
			n-Pentadecanoic acid	$C_{15}H_{30}O_2$	242.0	69.6	Traces
			Hexadecanoic acid (Palmatic)	$C_{16}H_{32}O_2$	256.4	64	Major
	Resins	–	Coloured resinous products				

It is now clear that because of its nature and complicated interconnections, the primary wall needs to be removed during cotton scouring. To minimise the number of different enzymes involved in the scouring process, it is desirable to select one that is most suitable for destabilisation of the primary wall. From the discussion on interconnections in the primary wall, it is clear that pectin is one of the most complicated non-cellulosic constituents in the primary wall. Grant [33] reported that pectins act as cementing material that contributes substantially to the hydrophobicity of cotton fibre. According to Thakur *et al.* [30] pectins can be classified into neutral (esterified pectins) and acidic (non-esterified) pectins. Esterified pectins prevent Ca^{2+}-induced bond formation and are located at the outer layer of the primary cell wall. Esterified pectins are necessary for the cell wall expansion during the growth of the cotton fibre [30]. Alkaline pH facilitates the degradation of esterified pectins via the β-elimination cleavage of glycosidic bonds [31]. Therefore, we postulated that alkaline hydrolytic pectinases that can degrade these esterified pectins would be desirable. The winding layer, a layer between the primary and secondary wall of a cotton fibre, contains non-esterified pectins. Ca^{2+} ions present in the winding layer cross-link the acidic pectins, thereby holding cell-wall components together. This bond formation between two non-esterified pectins is very strong and forms a rigid structure that ultimately leads to the prevention of secondary wall expansion [31].

From the overall discussion on cotton fibre structure it is clear that the waxy layer contributes significantly to the hydrophobicity of the cotton fibre. Scouring materials have to cross this first barrier in order to attack the primary wall components of the fibre. Therefore, we hypothesized that more attention should be paid to an additional step to remove the waxes prior to pectinase treatment. To allow pectinase to react efficiently with the substrate, the disruption and removal of the outermost waxy layer is important [43–45]. In this way maximum hydrophilicity is achieved at the first stages of the process before the pectinase incubation, instead of just treating the fabric with expensive enzymes. In this article, the effect of wax removal treatment is evaluated in terms of change in hydrophilicity and pectinase performance. Different acidic and alkaline pectinases have been evaluated for their scouring performance.

2 Materials and methods

2.1 Enzymes

The enzyme alkaline pectinase (EC 4.2.2.2) used in this study is BioPrep 3000L from Novozymes, Denmark. BioPrep 3000L contains a mono-component pectate lyase (PL) that is active in alkaline pH (pH 8–10). In commercial enzyme products, unknown stabilisers, emulsifiers and or surfactants are added to improve its performance. This complicates comparing other pectinases with BioPrep 3000L. Hence to remove stabilisers, precipitation of BioPrep 3000L was carried out with ammonium sulphate. Precipitation of BioPrep 3000L, and the production of a PL from *Bacillus pumilus* are described elsewhere [46, 47]. All three acidic pectinases are polygalacturonases (PG) and were isolated from the fungal strains of *Sclerotiums rolfsi* [48]. Two different media, glucose and cellulose, were tested for PG production. Finally, three different PGs (PG-glucose, PG-cellulose, and PG-precipitated) were used for the scouring experiments.

2.2 Fabrics

The fabric used was an industrially desized 280 g/m^2 plain-woven 100% cotton fabric, supplied by Ten Cate Technical Fabrics B.V. in the Netherlands. The pore volume distribution (PVD) for a Ten Cate industrially desized fabric was measured with an auto-porosimeter in receding mode (from low pressure to high pressure) to eliminate the effect of irregular or interconnected pores. To check the homogeneity of the fabric two samples of 6 × 6 cm were collected diagonally from the randomly selected 12 × 12 cm fabric and a PVD analysis was performed. NaOH scoured cloth was prepared in batch mode, resembling industrial process conditions (0.25 M NaOH in presence of commercial scouring additives at 95°C for 180 min) and supplied by TNO Science and Industry in the Netherlands. All experiments were performed with demineralised water.

2.3 Structural contact angle

To study the change in hydrophilicity (wetting) of a fabric sample, two methods are widely used, the so-called 'standard drop test' [49] and the 'water absorption test'. Both tests are greatly affected by changes in pore size resulting from different treatments. That is why 'drop test' values give large standard deviations. Moreover, in both tests, changes in PVD cannot be monitored; hence the results might be difficult to interpret. Therefore, a TRI auto-porosimeter [50] was used here to measure the structural contact angle of fabric together with information on the inter- and intra-yarn pore size distribution. The pore size is determined via its effective radius, and the contribution of each pore size to the total free volume. The advantage of an auto-porosimeter above other wettability experiments is that the results obtained are independent of the fabric density, and of the structure of the fabric (*e.g.* knitted or woven).

Miller *et al.* [50] described the operating procedure for liquid porosimetry in detail, which requires quantitative monitoring of the movement of liquid into or out of a porous structure. The effective radius R_{eff} of a pore is defined by the Laplace equation. The structural contact angle θ within a porous network can be determined from two plots of the liquid uptake *vs.* the effective radius. The first measurement is done with a test liquid (double-distilled water) and the other with a reference liquid (0.1% Triton X-100, a non-ionic surfactant solution). It is assumed that $\cos(\theta)_{ref} = 1$ for the reference liquid and that the test liquid does not form a zero contact angle. In that case, it is possible to calculate $\cos(\theta)_{test}$ value for the test liquid by:

$$\cos(\theta)_{test} = \left(\frac{2\gamma_{ref}}{\Delta P_{ref}}\right) \Big/ \left(\frac{2\gamma_{test}}{\Delta P_{test}}\right)$$

Where γ is the surface tension (mN/m), and ΔP is the Laplace pressure difference across the liquid/air meniscus (Pa). It should be kept in mind that for this equation to hold, the porous structure of the specimen must be unchanged during switching from one liquid to the other.

2.4 Pectin removal

Residual pectin on the fabric was analysed by staining it with ruthenium red [7]. This method is well adopted to evaluate pectin removal in scouring [13, 15, 45, 47, 51]. Briefly, two fabric samples of which the pectin content has to be determined were sewed together with the grey fabric (100% pectin control) and an alkaline scoured fabric (0% pectin control) to form a circular shaped fabric with a diameter of 8 cm. The fabric was then dyed with

ruthenium red dye solution (100 mL/g fabric) for 15 min at room temperature and rinsed afterwards with 1 L demineralised water. Each swatch was then washed with demineralised water for 10 min at 60°C in a Linitester. After line-drying overnight, the reflectance at 540 nm was measured and K/S values were calculated. The K/S value is a number related to the amount of the dyestuff present in a substrate, using the Kubelka-Munk equation [7]. From these values, the percentage of residual pectin was determined using controls [7, 51]. The average SD in the pectin removal values was found to be 0.5–6%. One litre of ruthenium red dye solution contained the following components: 0.2 g ruthenium red (Aldrich), 1.0 g ammonium chloride, 2.5 mL ammonium hydroxide solution (28%), 1.0 g Silwet L-77 (Witco Europe SA, Geneva, Switzerland) and 1.0 g Tergitol 15-S-12 (Aldrich). The purpose of both Silwet L-77 and non-ionic surfactant Tergitol 15-S-12 is to facilitate wetting of ruthenium red on the surface of cotton fabric for uniform dyeing.

2.5 The general experimental setup for scouring experiments

To benchmark the effect of wax removal, desized fabric was extracted with *n*-hexane in a Soxhlet apparatus for 30 min at 75°C [43, 44, 45]. It is assumed that all surface waxes are removed by the solvent extraction [52]. Scouring experiments where performed in an 1-L beaker in which three fabric samples of 5 × 12 cm were treated in an enzyme solution of 500 mL, including the 50 mM Tris-HCl buffer (pH 8). The beaker was placed in a temperature controlled water bath at 30 or 50°C. The applied shear was minimum (100 rpm), just enough to keep the fabric sample suspended in the reaction vessel. After the treatment, the fabric samples were rinsed in 500 mL water at 90°C for 15 min, to inactivate the enzymes. Thereafter, the samples were rinsed twice for 5 min in water at room temperature. Finally, the samples were kept on a flat acrylic surface for air drying at least 24 h before evaluating the fabric samples. All the experiments were performed in duplicate. Other details or modifications of this protocol are explained in the corresponding paragraphs.

3 Results and discussion

3.1 Uniformity of cotton fabrics – a substrate selection

For proper benchmarking of the existing scouring process in terms of hydrophilicity, the fabric substrate should be relatively uniform in terms of its PVD. A proper determination of PVD for woven cotton fabric is a difficult task, since the pores in cotton fabric are irregular in shape and may be interconnect to each other. The results from PVD measurements with the TRI auto-porosimeter are shown in Figure 2. From this figure it is apparent that most of the intra-yarn pores are in the range of 3–8 μm, while the inter-yarn pores are frequently observed in the range of 38–57 μm, with a mean radius of 46 μm. Both the PVD distributions match very well and are in the same range for both the intra-yarn and the inter-yarn pores.

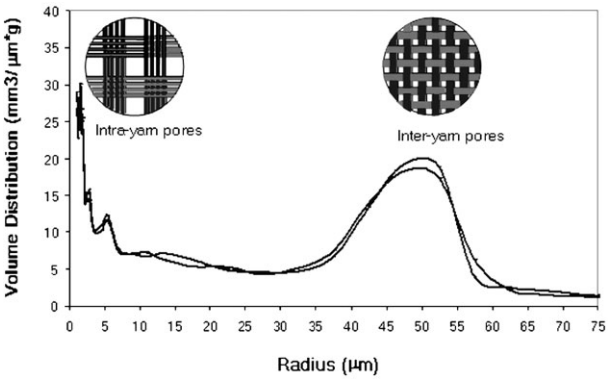

Figure 2. A PVD analysis for the Ten Cate industrially desized fabric (~280 g/m^2) in receding mode using Triton X-100 (0.1 g/L).

3.2 Benchmarking

Proper benchmarking of the current alkaline scouring is essential to establish a clear relationship with other treatments. Miller *et al.* [50] showed that the structural contact angle θ is directly proportional to the hydrophobicity of cotton fibre. Therefore, the structural contact angle and pectin removal have been measured for untreated fabric, $\theta_{untreated}$, after the wax removal, $\theta_{primary\ wall}$, and after scouring with NaOH, $\theta_{scoured}$ fabric. The contact angles for untreated fabric, primary wall and scoured fabric are in the following order $\theta_{untreated} > \theta_{primary\ wall} > \theta_{scoured}$ [10, 50]. The structural contact angle was used to benchmark the performance of the current alkaline scouring process and to compare the results of other treatments. The results of the treatments are shown in Figure 3. Sample A was the blank that was not treated at all. Sample B was extracted with *n*-hexane to remove the waxes. Sample C was scoured conventionally with NaOH. The structural contact angle of untreated cotton was 83°, the structural contact angle after solvent extraction was 68° and the structural contact angle of the conventionally scoured fabric was 53°. This latter value is thus the one must be achieved in a new scouring process. The average SD in the structural contact angle measurements was in between 3° and 7°. As shown in Figure 3, pectin removal for untreated fabric was considered as 0% and for the NaOH scoured fabric 100%. Solvent extraction treatment targeted to the wax removal, as a result, less than 7% pectin was removed (Fig. 3).

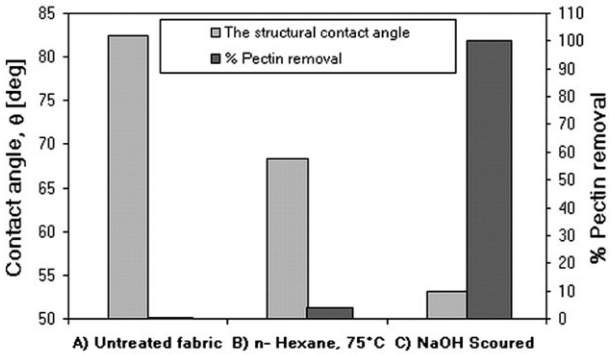

Figure 3. Change in the structural contact angle and percentage pectin removal of fabric after different treatments. (A) Standard fabric as a blank sample, (B) fabric extraction with *n*-hexane for 30 min at 75°C to remove surface waxes and (C) NaOH-scoured sample as a benchmark.

3.3 Potential of different pectinases

Effects of six selected pectinases (three different acidic PGs and three alkaline PLs) for pectin removal were studied. To be sure that only the enzymatic effect of pectinase was determined, all fabric samples used have been treated first with *n*-hexane at 75°C for 30 min. It was assumed that this removed the wax layer and that the primary wall that contains the pectin layer was then fully exposed to the enzymes. The concentration of all the pectinases used was 100 U/g fabric. Experiments with acidic PGs were performed at pH 5, whereas for the alkaline PLs the recommended pH 8 was used. All the treatments lasted 30 min at 50°C (recommended temperature). Figure 4A shows the results obtained with the acidic pectinases and Figure 4B shows the results with the alkaline pectinase treatments.

The acidic PGs remove 45–52% of the pectin. Pectinases produced on a cellulose-containing medium contained some low endoglucanse activity in contrast to pectinases produce on glucose medium. This obviously enhances the effect of pectinases probably by improving accessibility of the substrate to the enzyme [48]. In contrast, with the alkaline pectinases (PL or BioPrep 3000L), 76–83% of the pectin was removed. This means that the performance of alkaline pectinases is approximately 75% higher compared to the acidic pectinases (PGs). As far as the structural contact angle θ is concerned, the treatment with PGs gave a contact angle of ~66° that was higher than with alkaline pectinases θ ~60°. Blank samples without any pectinase treatment had a structural contact angle of ~69°. Figure 4B shows that there was almost no difference between the performances of the three selected alkaline pectinases in terms of the structural contact angle and pectin removal. Three conclusions can be drawn here; firstly, the performance of the alkaline pectinase is better than acidic PGs. Secondly, the pectinolytic capability of alkaline PL from *B. Pumilus* is equivalent to that of commercial BioPrep 3000L and, finally, the precipitated BioPrep 3000L has a similar performance as commercial BioPrep 3000L.

The enzymatic mechanisms of acidic PG and alkaline PL are explained in literature [46, 47]. The PG cleaves the galacturonic acid by an acidic hydrolysis reaction, while PL degrades pectin by a β-elimination reaction. The same authors found that PG cleaves α-1-4 glycosidic linkages in pectin that are not esterified (acidic pectins). PL cleaves the partially esterified pectin backbone and the long methylated chains of rhamnogalacturonan, which results in the formation of a double bond between C4 and C5 at the non-reducing end.

Krall *et al.* [53] reported that the degree of pectin esterification has an opposite effect on the rate of degradation by acid hydrolysis compared to its effect on the rate of β-elimination. Alkaline pH also promotes the β-elimination reaction and the de-esterification process of methyl-esterified pectin. These two mechanisms help to break the pectin polymer interaction between two esterified pectin molecules at the outer surface of the cotton fibre [34]. The presence of partially esterified pectins on the outer layers of the cotton fibre and the alkaline pH in-

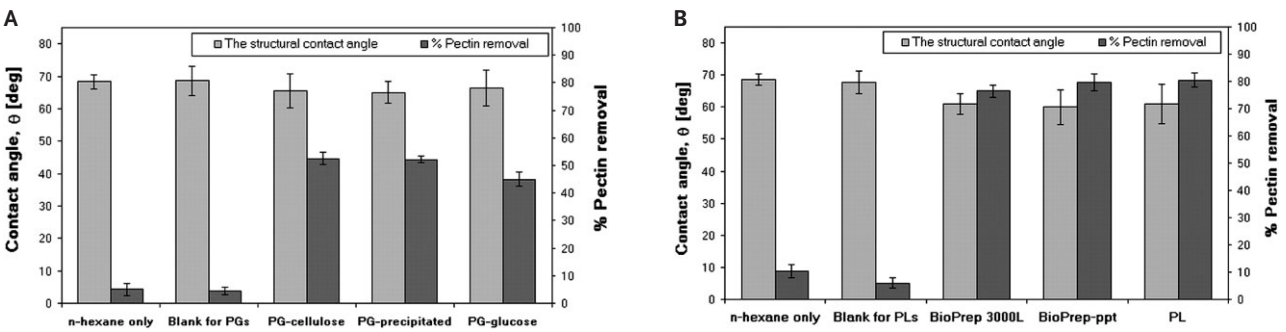

Figure 4. Effect of six different pectinases on the structural contact angle and percentage pectin removal. (A) Ten Cate industrially desized fabric (~280 g/m^2) samples were treated with three different acidic pectinases (PGs) at pH 5, in 50 mM acetate buffer, (B) fabric samples were treated with BioPrep 3000L, precipitated BioPrep 3000L and PL from *B. Pumilus* in 50 mM Tris-HCl buffer, pH 8. All experiments were performed at 50°C, for 30 min using 100 U/g pectinase.

creases the performance of alkaline pectinase compared to acidic pectinase [45]. The obtained results prove our postulation about the superior performance of alkaline pectinase on cotton pectin. From this point on, PL an alkaline pectinase was used in all other experiments and is subsequently named 'pectinase' here.

3.4 Wax removal

To verify our stated hypothesis, the effect of the waxy layer on pectinase performance towards improved hydrophilicity was evaluated. The aim was to determine whether wax removal or the presence of surfactants improves the performance of pectinase. Two different sets of experiments were done with the standard fabric. In the first set, various treatments were given to the fabric followed by a pectinase treatment. It has been deduced that boiling with water or buffer solution would melt some of the cotton waxes, resulting in an improved pectinase penetration in the primary wall [10]. These different treatments were: (a) blank or untreated fabric, (b) boiling with demineralised water at 100°C for 2 min, (c) boiling with 50 mM, Tris-HCL buffer, pH 8 at 100°C for 2 min and (d) *n*-hexane extraction 75°C for 30 min. In the second set of experiments the same treatments (a–d) were given to affect or remove waxy layer, but this time the pectinase treatment was done in presence of 1 g/L Triton X-100. The compatibility of Triton X-100 with pectinases was confirmed by conducting the activity assay [45].

Figure 5A shows that a treatment to remove waxes has a positive influence on the structural contact angle. However, the change in the structural contact angle was small, θ ~77° for the untreated fabric and θ ~68° with *n*-hexane treatment (both blank experiments). Treatment with demineralised water (b) and buffer-treated fabric (c) showed almost the same structural contact angle θ ~71°. Figure 5A shows that the difference in structural contact angle is maximum for *n*-hexane-extracted fabric (θ difference >7°), compared to any other treatment. The decrease in θ for the solvent-extracted fabric can be ascribed to an increased surface area accessible for pectinase treatment. Results from the second set of experiments are similar to the first set except for the treatment with *n*-hexane followed by pectinase and Triton X-100 (Fig. 5B). For *n*-hexane-treated fabrics, a significant difference in θ, ~12°, was achieved comparing the blank with the pectinase-treated sample. A θ of ~54°, which is close to the desired value was obtained using surfactants in combination with BioPrep 3000L (Fig. 5B).

From Figure 5, it can be concluded that the incorporation of a treatment that removes surface waxes, like the *n*-hexane treatment, leads to an increased hydrophilicity of the fabric, making the pectin surface of the fabric more accessible to pectinase. Moreover, it can be concluded that, for *n*-hexane-treated fabrics, a non-ionic surfactant such as Triton X-100 has a positive impact on the pectinase performance because of the better surfactant penetration inside the yarns. Therefore, the principal challenge in enzymatic cotton scouring is not just the degradation of pectin, but to increase hydrophilicity by removing cotton waxes at low temperatures effectively and efficiently via a benign route. This topic is discussed elsewhere in detail ([45], and Agrawal *et al.,* 2006 to be published).

3.5 Pectin removal

In Section 3.4, it was demonstrated that a pectinase treatment after wax removal leads to an improved hydrophilicity. Therefore, the effect of wax removal on pectinase hydrolytic rate was evaluated. We hypothesised that wax removal from the cotton fibre surface would result in more pectin surface available for the pectinase action, and hence the kinetics of pectin hydrolysis will be faster. The evaluation was done in terms of pectin removal as a function of time for different treatments. The standard fabric was considered as a control sample with an intact

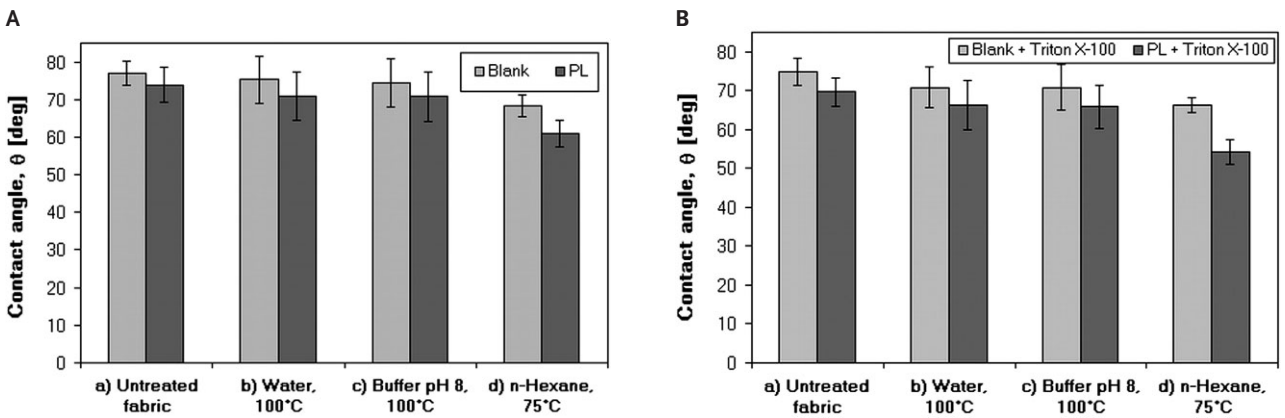

Figure 5. Effect of various treatments to affect or remove waxes on pectinase performance in terms of the structural contact angle. Various treatments followed by 100 U/g PL incubation at 50°C for 30 min, in 50 mM Tris-HCl buffer, pH 8, and the appropriate blanks. (A) Treatment followed by blank and alkaline pectinase (PL) incubation, (B) treatment followed blank and alkaline pectinase (PL) incubation in presence of 1 g/L Triton X-100.

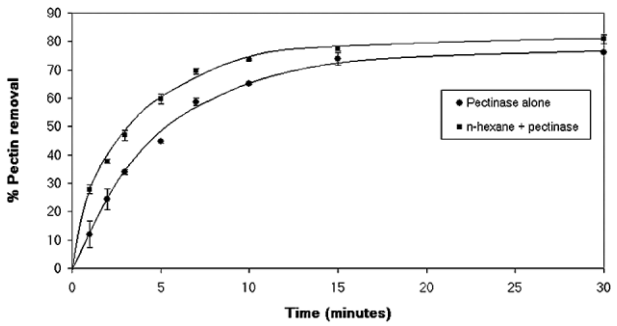

Figure 6. Performance of PL (13 U/g fabric) in terms of pectin removal before (●), and after wax extraction (■). Lines are tentatively drawn. Desized fabric was subjected to *n*-hexane extraction at 75°C for 30 min prior to pectinase incubation.

waxy layer on its fibre surface. Another sample was the *n*-hexane extracted fabric followed by the pectinase treatment.

From Figure 6 it is clear that the kinetics of pectin hydrolysis was faster for the fabric sample that was previously treated with the *n*-hexane. The rate of pectin hydrolysis followed first order kinetics. After 30 min of pectinase incubation, 76–80% of pectin was removed. The time constant for these two different treatments was calculated by the 63% method [45]. Pectinase treatment alone takes nearly 9 min to remove 63% pectin; however, *n*-hexane treatment followed by pectinase needed only 5.5 min for the same amount of pectin removal. Therefore, the initial rate of pectin hydrolysis for the *n*-hexane extracted fabric is nearly 1.65 times higher than the pectinase treatment alone.

In our experiments the substrate was not varied for the two treatments; hence, the rate of pectin hydrolysis is completely dependent on the available substrate for pectinase action. *n*-Hexane extraction of the fabric resulted in larger surface area, which facilitates exposure of non-cellulosic interconnections in the primary wall to the pectinase.

Interestingly, from the structural contact angle measurements (Fig. 5A), the θ was ~74° for pectinase-only-treated fabric, and ~62° for fabric that was first treated with *n*-hexane followed by pectinase; a difference of ~12°. However, the amount of pectin removal is nearly the same (76–80%) with both treatments after 30 min. Therefore, it can be concluded that the structural contact angle is influenced by both wax and pectin removal. In case of *n*-hexane extraction followed by a pectinase treatment, the waxes are removed efficiently, but that was not the case with the pectinase-only treatment. To conclude, the addition of an efficient wax removal step improves the performance of pectinase in terms of pectin removal and hydrophilicity of the fabric.

4 Concluding remarks

A systematic approach is necessary to design and introduce an adequate continuous bioscouring process. A thorough literature survey reveals many clues, which have direct influence on designing an efficient enzymatic scouring process. Wax and pectin removal are identified to be the most important steps in the scouring process. A TRI auto-porosimeter was used to measure the structural contact angle of the fabrics. Benchmarking of the existing NaOH scouring process was done by measuring the structural contact angle θ and pectin removal for untreated ($\theta_{untreated}$), after wax removal ($\theta_{primary\ wall}$), and for alkaline scoured fabric ($\theta_{scoured}$). A structural contact angle of less than 53° is needed to achieve sufficient hydrophilicity in the scouring process.

Six selected acidic and alkaline pectinases were tested for their scouring potential after wax removal. The difference in performance can be deduced from the cotton structure. The pectin removal performance of alkaline pectate lyases (PL and BioPrep 3000L) is nearly 75% higher than that of acidic polygalacturonases (PG). Moreover, alkaline pectinase improves the hydrophilicity (θ~60°) more than the acidic pectinase (θ≈66°). The specially produced PL from *B. Pumilus* was, like the commercial BioPrep 3000L, a potential enzyme for an industrial bioscouring process.

Results validate the hypothesis that the disruption and removal of the outermost waxy layer is of prime importance to allow pectinase to react efficiently with the substrate. This should result in a more efficient process because of improved hydrophilicity. *n*-Hexane-extracted fabrics followed by the pectinase incubation in the presence of surfactant produces the best results, θ~54°, which was close to that of alkaline scoured fabric. The rate of pectin hydrolysis was nearly 1.65 times faster when the fabric was treated with *n*-hexane followed by the pectinase. From these results it was concluded that wax removal is a prerequisite for higher pectinase performance to achieve sufficient hydrophilicity in the enzymatic scouring process.

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5 References

[1] Throtman, E. R. (Ed.), *Dyeing and Chemical Technology of Textile Fibre*, 6th edn, Charles Griffin, London 1984.

[2] Karmakar, S. R. (Ed.), *Chemical Technology in the Pretreatment Processes of Textiles*. *Textile Science and Technology Series*, Vol. 12, Elesevier Science B. V., Amsterdam 1999.

[3] Rouette, H. K. (Ed.), *Encyclopaedia of Textile Finishing*, Vols. 1–3, Springer, Berlin 2001.

[4] Nierstrasz, V. A., Warmoeskerken, M. M. C. G., Process engineering and industrial enzyme applications, in: Cavaco-Paulo A., Gübitz, G. M. (Eds.), *Textile Processing with Enzymes*, Woodhead Publishing, Cambridge 2003, pp. 120–154.

[5] Lin, C.-H., Hsieh, Y.-L., Direct scouring of greige cotton fabric with proteases. *Textile Res. J.* 2001, *71*, 415–434.

[6] Li, Y., Hardin, I. R., Enzymatic scouring of cotton: effects on structure and properties. *Text. Chem. Color.* 1997, *29,* 71–76.

[7] Lange, N. K., Liu, J., Husain, P., Condon, B., *Book of papers on the International Conference and Exhibition of the AATCC*, Philadelphia, USA, September 22–25, 1998, pp. 463–471.

[8] Sawada, K., Toniko, S., Ueda, M., Wang, X. Y., Bioscouring of cotton with pectinase enzyme. *JSDC* 1998, *114*, 333–336.

[9] Etters, J. N., Husain, P. A., Lange, N. K., Alkaline pectinase: an eco friendly approach to cotton preparation. *Text Asia.* 1999, *5,* 83–86.

[10] Hartzell-Lawson, M. M., Hsieh, Y-L., Enzymatic scouring to improve cotton fabric wettability. *Textile Res. J.* 1998, *68,* 233–241.

[11] Buchert, J., Pere, J., Scouring of cotton with pectinases, proteases and lipases. *Text. Chem. Color AM. D.* 2000, *31,* 48–52.

[12] Tzanko, T., Calafell, M., Guebitz, G. M., Cavaco-Paulo, A., Bio-preparation of cotton fabrics. *Enzyme Microb. Technol.* 2001, *29,* 357–362.

[13] Takagishi, T., Yamamoto, R., Kikuyam, K., Arakawa, H., Design and application of continuous bioscouring machine. *AATCC Rev.* 2001, *1,* 32–35.

[14] Degani, O., Gepstein, S., Dosoretz, C. G., Potential use of cutinase in enzymatic scouring of cotton fibre cuticle. *Appl. Biochem. Biotechnol.* 2002, *102,* 277–290.

[15] Lenting, H. B. M., Zwier, E., Nierstrasz, V. A., Identifying important parameters for a continuous bioscouring process. *Textile Res. J.* 2002, *72,* 825–831.

[16] Calafell, M., Garriaga, P., Effect of some process parameters in the enzymatic scouring of cotton using an acid pectinase. *Enzyme Microb. Technol.* 2004, *34,* 326–331.

[17] Lu, H., Insights into cotton enzymatic pretreatment. *Int. Dyer.* 2005, *190,* 10–13.

[18] Csiszár, E. Szakács, G., Oxidative enzymes in biopreparation of cotton and linen fabrics. *Proceedings of the 4th international conference on Textile Biotechnology*, Seoul, Korea, Book I, June 4–7, 2006, p. 40.

[19] Fan, X., Wang, Q., Wang, P., Gao, W., Study on the enzymatic scouring of linen/cotton fabrics. *Proceedings of the 4th international conference on Textile Biotechnology*, Seoul, Korea, Book I, June 4–7, 2006, p. 57.

[20] Levy, O., Bio-preparation – A new approach in cotton scouring. *Int. Dyer.* 2006, *191,* 11–12.

[21] Luis J. del V., Marta, O., Pere, G., Margarita, C. Schnitzhofer W., Guebitz G. M., Bioscouring of cotton fibre with polygalacturonase induced in Sclerotium rolfsii using cellulose and glucose pectin. *Textile Res. J.* 2006, *76 ,* 400–405.

[22] Moghe, V. V., Nabar, P. S., Bio scouring: An ecological way of scouring. *Colourage* 2006, *53,* 95–96.

[23] Shin, H.-J., Bioscouring of cotton fabrics by extracellular pectinase from Aspergillus sp. *Proceedings of the 4th international conference on Textile Biotechnology*, Seoul, Korea, Book I, June 4–7, 2006, p. 46.

[24] Csiszár, E., Szakács, G., Rusznák, I., Combining traditional cotton scouring with cellulase enzymatic treatment. *Textile Res. J.* 1998, *68,* 163–167.

[25] Ryser, U., Holloway, P. J., Ultrastructure and chemistry of soluble and polymeric lipids in cell walls from seed coats and fibres of *gossypium species*. *Planta* 1985, *163*, 151163.

[26] Ryser, U., Meier, H., Holloway, P. J., Identification and localization of suberin in the cell walls of green cotton fibres (*Gossypium hirsutum* l., var. green lint). *Protoplasma* 1983, *117*, 196205.

[27] Varadarajan, P. V., Iyer, V., Saxana, S., Wax on cotton fibre: its nature and distribution – a review. *Indian Soc. Cotton Improv. J.* 1990, *15,* 453–457.

[28] Schmutz, A., Buchala, A., Jenny, T., Ryser, U., The phenols in the wax and the suberin polymer of green cotton fibres and their functions. *Acta Hortic.* 1994, *1,* 269–275.

[29] Kerstiens, G. (Ed.), *Plant cuticles an integrated functional approach*. *Environmental Plant Biology Series*, BIOS Science, Oxford 1996.

[30] Thakur, B. R., Singh, R. K., Handa, A. K., Chemistry and uses of pectin – a review. *Crit. Rev. Food Sci.* 1997, *37,* 47–73.

[31] Vughn, K. C., Turley, R. B., The primary wall of cotton fibre contains and ensheathing pectin layer. *Protoplasma* 1998, *209,* 226–237.

[32] Hartzell-Lawson, M. M., Hsieh, Y-L., Characterising the noncellulosics in developing cotton fibres. *Textile Res. J.* 2000, *70,* 810–819.

[33] Grant, R. J. S., Cementing the wall: cell wall polysaccharide synthesizing enzymes. *Curr. Opin. Plant Biol.* 2000, *3,* 512–516.

[34] Ridley, B. L., Malcom, A. O., Mohnen, D., Pectins: structure, biosynthesis and oligogalacturonide-related signaling – a review. *Phytochemistry* 2001, *57,* 927–967.

[35] Warwicker, J. O., Jeffries, R., Colbran, R. L. (Ed.), *A review of the literature on the effect of caustic soda and other swelling agents on the fine structure of cotton*. *Shirly Institute Pamphlet*, No. 93, Didbury, Manchester 1966.

[36] Cutler, D. F., Alvin, K. E., Price, C. E. (Eds.), *The plant cuticle*, Academic Press, London 1982.

[37] Croteau, R., Kolattukudy, P. E., Enzymatic biosynthesis of a hydroxy fatty acid polymer, cutin, by a particulate preparation from vicia fada epidermis. *Biochem. Biophys. Res. Commun.* 1973, *52,* 863–869.

[38] Kolattukudy, P. E., Structure, biosynthesis, and biodegradation of cutin and suberin. *Am. Rev. Plant Physiol.* 1981, *32,* 539–567.

[39] Luque, P., Bruque, S., Heredia, A., Water permeability of isolated cuticular membranes: a structural analysis. *Arch. Biochem. Biophys.* 1995, *317,* 417–422.

[40] Albert B., Dennice, B., Julian, L., Martin, R. *et al.,* (Eds.), *Molecular Biology of the Cell*, 2nd edn., Garland Publishing, New York 1989.

[41] Darnell, J., Lodish, H. D., Baltimore, A., Berk, S. L. *et al.,* (Eds.), *Molecular Cell Biology,* 3rd edn., Scientific American Books, New York 1995.

[42] Jocelyan, K. C. R., Bennett, A. B., Cooperative disassembly of the cellulose-xyloglucan network of plant cell walls: parallels between cell expansion and fruit ripening. *Trends Plant Sci.* 1999, *4,* 176–183.

[43] Agrawal, P. B., Nierstrasz, V. A., Warmoeskerken, M. M. C. G., Enhanced bioscouring performance. *Proceedings of the annual international conference and exhibition of AATCC*, Section 7, September 9–12, 2003a, pp. 165–173.

[44] Agrawal, P. B., Nierstrasz, V. A., Warmoeskerken, M. M. C. G., Does and don'ts in bioscouring: opportunities for an efficient enzymatic scouring process. *Proceedings of the 3rd AUTEX Conference*, Gdansk, Poland, Book I, June 25–27, 2003b, 82–88.

[45] Agrawal, P. B., *The performance of cutinase and pectinase in cotton scouring*. PhD thesis, University of Twente, The Netherlands, ISBN 90-365-2243-9, 2005.

[46] Klug, B. G., 2004. A new pectate lyase from *Bacillus pumilus*, Doctoral thesis, Graz University of Technology, Graz, Austria.
[47] Klug-Santner, B. G., Schnitzhofer, W., Vršanská, M., Weber, J. *et al.,* Purification and characterization of a new bioscouring pectate lyase from *Bacillus pumilus* BK2. *J. Biotechnol.* 2006, *121,* 390–401.
[48] Schnitzhofer, W., Kandelbauer, A., Klug-Santner, B., Onos, M. *et al.,* Production of cellulase-free polygalacturonase preparation by *Sclerotium rolfsii* for bioscouring of cotton. *J. Nat. Fibres.* (in press).
[49] American Association of Textile Chemist and Colorist (AATCC), *Technical manual*, 1997, *27,* 286.
[50] Miller, B., Tyomkin, I., Liquid porosimetry: new methodology and applications. *J. Colloid Interf. Sci.* 1994, *162,* 163–170.
[51] Novozymes, Determination of pectin removal on cotton by ruthenium red dyeing. SOP No.: EUS-SM-0103, 02/01 1-6, 2003.
[52] Walton, T. J., Waxes, cutin and suberin. *Methods Plant. Biochem.* 1990, *4,* 105–158.
[53] Krall, S. M., McFeeters, R. F., Pectin hydrolysis: effect of temperature, degree of methylation, pH, and calcium on hydrolysis rates. *J. Agric. Food Chem.* 1998, *46,* 1311–1315.