A SUSTAINABLE APPROACH FOR COTTON BIOSCOURING: REUSE OF THE PECTATE LYASE CONTAINING TREATMENT BATH

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ABSTRACT

Enzymatic scouring of cotton has established itself (slowly) as a green alternative to alkaline scouring in the textile industry, mostly due to more environmentally friendly processing at lower pH and temperatures and its less aggressive action on the cotton fibers. However, amongst other limitations enzyme costs have contributed to impeding its wide acceptance and use. For the first time, in this study, the recycling of the bioscouring bath was evaluated, unlike most current bioscouring that is performed using fresh enzyme solution. Bioscouring of raw knitted cotton fabric was carried out for 30 minutes with a commercial pectinase (BioPrep® 3000L) at 55 °C and pH 8.5. About 89 % of the recovered pectate lyase-containing scouring

bath was completed with 11 % of fresh enzyme solution and reused in a new bioscouring process under the same conditions. Up to ten reuse cycles were possible maintaining the level of pectin removal and without significant loss in quality of subsequent dyeing. A detailed analysis of the pretreated fabrics is presented. Reusing the scouring bath, reducing the intensive consumption of input materials (enzyme, water, and chemicals) and wastewater generation can be possible, making bioscouring a more attractive and sustainable technique. The process demonstrated is promising and its industrial application is feasible.

Keywords: Enzymatic scouring. Enzyme reuse. Water economy. Ecological process. Textile industry.

1. INTRODUCTION

Cotton is an important natural resource and one of the most used materials in the textile and clothing industry [1]. Cotton fiber is a highly pure cellulose deposit and consist of four concentric layers in its morphological structure from the outside to the inside: (1) the cuticle, a thin outer membrane composed of waxes, proteins, and pectins; (2) the 0.1 microns thick primary wall, consisting of 52 % cellulose with a complex mixture of pectins, waxes, proteins, ash, and other organic compounds comprising the rest; (3) the secondary wall, which is the thickest layer formed by parallel and overlapping celluloses; and (4) the lumen, a central channel composed of protoplasmic residues [2, 3]. The amount of non-cellulosic impurities represents about 4-12 % of the total mass and is responsible for the hydrophobic properties of raw cotton and creating difficulties in textile dyeing [4]. A cleaning step for the removal of these impurities is necessary before dyeing, printing, and finishing [5].

Alkaline scouring is the conventional preparation step to remove non-cellulosic constituents on raw cotton and involves a treatment with a hot solution (90-100 °C) of sodium hydroxide (NaOH, 1 mol/l) and other chemicals for up to one hour, accompanied by subsequent washing steps [6]. The result is an almost pure cellulose fiber (content is over 99 %) with excellent water absorbency [4]. Although alkaline scouring is effective and the costs of NaOH are low, it carries an environmental burden and represents one of the most aggressive steps in textile processing, because it consumes large quantities of chemicals, water, and energy [7, 8] . Depending on operation conditions, attack of the cellulosic component of cotton and reduced physical strength of the fibers and have been reported [9-11]. Scouring with strong alkali (NaOH) at high temperatures and in the presence of oxygen may result in serious shrinkage, swelling, and oxidation of cellulose leading to fiber damage and affecting the mechanical properties of cotton fabric [12].

Scouring with biodegradable enzymes at moderate temperatures and pH has proved to be a sustainable alternative and has been widely accepted in the industry, however, some drawbacks such as high enzyme costs and a lower whiteness degree still have to be overcome [1]. Bioscouring with pectinases alone or associated with proteases, lipases, cutinases, and cellulases have been applied under smooth reaction conditions and with minimal damage to the cellulosic structure [13-22].

Pectinases, a group of enzymes that degrade pectin substances, are the most promising biocatalysts for bioscouring [7]. Pectin is a complex heteropolysaccharide consisting primarily of 65 % galacturonic acid residues joined via α(1→4) linkages in a linear pattern, with sections containing branch points which side chains of arabinose and galactose are predominate [23]. The high methylation (85 %) of the polygalacturonic acid groups in the pectins leads to highly hydrophobic pectin, contributing to the lack of water absorption of raw cotton [3]. Pectinases

attack the polygalacturonic acid backbone, breaking it down by hydrolysis or beta-elimination, with no decomposition of the cellulose fibers which is an undesirable side activity of alkaline scouring [9]. Acid, alkaline, and neutral pectinases are usually applied [24] at temperatures between 50-60 °C and for 30 to 60 minutes [5]. After breaking and removing the pectin, which acts as a cementation adhesive barrier between cellulosic and non-cellulosic components [2, 25] , waxes and all other non-cellulosic material can be removed more easily using hot water and surfactants [14, 17] .

The limiting factor for the implementation of enzyme-based processes in the textile industry is related to the economic viability, mainly due to the high cost of enzymes [20]. According to Madhu and Chakraborty [25], recycling and reuse of enzymes would make the process more economically feasible and facilitate the replacement of harsh chemicals in the preparation of cotton. Considering the cost-related limitations of enzyme processes two different approaches can be made to lower the impact of enzyme cost on process costs: 1. Immobilization of the enzyme and its reuse 2. Recycling and reuse of the whole enzyme liquor. Immobilization of enzymes usually increases the cost of the biocatalyst and may result in reduced activity which has to be compensated by repeated use. Immobilization is however a tricky issue if the enzyme acts on a solid substrate [1].

Reducing water consumption is another key strategy in sustainable development to alleviate the environmental impact of the textile processing industry, which uses large amounts of water [26] and contributes significantly to water pollution, putting a great strain on global water resources [27, 28]. It is estimated that the textile industry uses more water than any other industry globally and that almost all wastewater discharged is highly polluted [29]. Average-sized mills consume about 200 l water per kg fabric [28, 29] , but depending on the production process water consumption can rise to 700 l per 1 kg of fabric [30]. Saving water means less

generation of effluent [26] and, in comparison to alkaline scouring, the enzymatic scouring can reduce rinse water consumption by 20-50 % [31]. Conserving water and mitigating water pollution is part of the textile industry's strategy to make its production processes more environmentally friendly, especially in parts of the world where water is scarce [31].

The main objective of this study was to evaluate the reuse of the scouring bath of a previously optimized bioscouring procedure with a commercial pectate lyase [17] up to ten times in enzymatic scouring of knitted cotton fabric and compare the obtained results to alkaline scouring. To our knowledge, based on recent literature searches in the specialized literature, this is the first report on the reuse of bioscouring baths in cotton scouring without immobilization of the enzyme.

2. MATERIAL AND METHODS

2.1 MATERIAL

The commercial enzyme BioPrep® 3000L ,an alkaline pectinase with pectate lyase (EC 4.2.2.2) activity from *Bacillus licheniformis*, (approximate density of 1.04 g mL^{-1} at 20 ºC) was kindly supplied by Novozymes®. The non-ionic surfactant Berol® 175 (C12-C16 alcohol ethoxylate 90 %, Nouryon, emulsifier, wetting agent) is based on natural primary alcohol and was kindly provided by Macler Produtos Químicos Ltda. Polygalacturonic acid was purchased from Sigma-Aldrich. The raw knitted 100 % cotton fabric (Jersey) (160 g/m²), made from 30.1 Ne combed yarns, was purchased from the local market. All chemical reagents were of analytical grade. In all experiments, distilled water was used unless indicated otherwise.

2.2 ENZYME ACTIVITY AND ENZYME STABILITY DURING THE INCUBATION PERIOD

Pectate lyase activity (EC 4.2.2.2) was determined according to Collmer, Ried, and Mount (1988) using a dilution of 1 mL L^{-1} of Bioprep 3000 L [32]. Protein concentration of

Bioprep 3000L was determined according to the Bradford method [33] as 34.73 ± 0.8613 g L^{1}. Polygalacturonic acid was used as the substrate. Substrate stock solution contained 50 mM Tris-HCl (tris(hydroxymethyl)aminomethane) buffer pH 8.5, 0.6 mM calcium chloride, and 0.24 % (w/v) polygalacturonic acid. Substrate stock solution (2.5 ml) and 0.5 ml of diluted enzyme solution (1 ml/l), both equilibrated at 55 °C, were rapidly mixed in a 1 cm cuvette and the increase in absorbance at 232 nm of the 4,5-unsaturated reaction products was monitored with a Shimadzu UV-1601 as a function of time so that the enzyme produced a linear rate of reaction for 30 seconds.

Enzyme activity was calculated according to equation (1), where: ΔAbs/Δt is the increase in absorbance of enzyme solution per time of incubation (min), ε is the molar extinction coefficient for the unsaturated products at 232 nm which is 4600 M^{-1} cm^{-1} [32], V_{total} is the total volume of the mixture (3.0 ml), V_{enzyme} is the volume of the enzyme (0.5 ml), and d is the factor of dilution of the enzyme before addition to the reaction (1000 times).

$$\frac{U}{ml} = \frac{\Delta Abs}{\Delta t} \times \frac{1}{\varepsilon} \times \frac{V_{total}}{V_{enzyme}} \times d \quad (1)$$

One unit of the enzyme (U) releases 1 μmol of 4,5-unsaturated pectic fragments per minute under the experimental conditions of the assay.

Enzyme stability was evaluated by incubating the enzyme at a dilution of 1.0 g/l corresponding to approximately 33,40 mg of protein) with the nonionic surfactant Berol® 175 (1.0 g/l) in 50 mM Tris-HCl buffer, pH 8.5, for 300 minutes at 55 °C. Enzymatic activity was measured every 30 minutes according to the procedure described above.

2.3 ENZYMATIC TREATMENT OF COTTON FABRICS – BIOSCOURING

Enzymatic treatments were carried out in a 500 ml steel vessel on a Kimak wash-tester (Kimak, Brusque, SC, Brazil) with 40 rpm vertical agitation. Raw cotton fabrics (5.0 g) were treated for 30 min at 55 °C and at a mass of fabric to liquor ratio of 1:20 (g:ml) with an aqueous

scouring bath of pH 8.5 (50 mM Tris-HCl buffer), containing 1.0 g/l pectinase BioPrep® 3000L and 1 g/l of non-ionic wetting agent (Berol® 175). After the treatment, each cotton fabric was removed from the bioscouring bath and washed with surfactant solution (0.2 g/l) at 70 °C at the same liquor ratio (1:20) for 10 min, rinsed extensively with tap water at room temperature and air-dried. The bioscouring bath was reserved for reuse. Control treatments were carried out similarly without the addition of enzymes. The process scheme is shown in Suppl Figure 1.

The bioscouring bath of the first cycle was completed with 11 ± 1 % of its original volume (100 mL) with a fresh enzyme solution containing the same ingredients and at the same concentrations of the first bioscouring bath, to make up for the loss of the liquid retained by the textile substrate in the first bioscouring cycle and then reused in a second bioscouring process. As the fresh enzyme solution (11 ± 1 mL) had the same enzyme concentration of the first scouring bath (0.2861 ± 0.057 U mL^{-1} or a total of 28.6 ± 0.57 U per 100 mL), approximately 3.15 U of fresh enzyme were added in each new cycle. Each bioscouring cycle was carried out following the protocol described above and was continued until completing ten consecutive cycles.

2.4 CONVENTIONAL SCOURING OF COTTON FABRICS

Conventional alkaline scouring (Suppl Figure 2) was carried out with sodium hydroxide, under conditions typically used in industries from Brazil [17][18]. A raw cotton fabric sample (approximate weight, 5.0 g) was immersed in sodium hydroxide solution (2.0 g/l) supplemented with a non-ionic wetting agent (surfactant Berol® 175, 1.0 g/l), and the mixture was incubated at 90 °C for 30 min. The liquor-to-fabric ratio was 1:20 (g:ml). To evaluate the efficiency of alkaline scouring, three additional and slightly different conditions for alkaline scouring were tested: 1. 2 g L^{-1} NaOH and 30 minutes at 95 ºC, 2. 4 g L^{-1} NaOH and 30 minutes at 95 ºC and 3. 2 g L^{-1} NaOH and 60 minutes at 95 ºC. Subsequently cotton fabrics were washed at 55 °C with distilled water for 1010 min, followed by a neutralizing wash with acetic acid (3.0 g/l) at

55 °C for another 10 min and finally exhaustively (approximately 10 minutes) rinsed with water until neutral pH.

2.5 DYEING

Scoured fabrics (1.0 g), without additional bleaching, were dyed at 60 °C for 60 minutes, using 1.7 % o.w.f. of Reactive Blue 222 (kindly provided by Siderquímica, São José dos Pinhais, PR, Brazil), at a liquor ratio of 1:20 (g:ml). Chemicals and auxiliaries were used according to the manufacturer's recommendations.

Aliquots of the dyeing bath were collected at regular time intervals, and the dye concentration remaining in the bath was determined at the maximum absorbance of λ 612 nm with a Shimadzu UV-1601 spectrophotometer.

2.6 TESTINGS

2.6.1 Weight loss

Fabric weight loss was expressed as a percentage with respect to the initial weight and calculated according to equation (2), where W_1 and W_2 correspond to the weights of the fabric before and after the scouring process, respectively. Before testing, all fabrics samples were conditioned at a relative humidity of 67 ± 2 %, in a controlled atmosphere above saturated copper chloride solution [34], for 24 h.

$$\text{Weight loss (\%)} = \left(\frac{W_1 - W_2}{W_1}\right) \times 100 \quad (2)$$

2.6.2 Whiteness, brightness, and yellowness

The whiteness index (Berger), brightness (ISO 2470 scale), and yellowness (ASTM-D-1925 scale) were obtained from reflection measurements with a benchtop spectrophotometer (CM-3610D, Konica Minolta). Berger Degree of whiteness is calculated from tristimulus values of the remission spectrum [34] according to:

$$W(\text{Berger}) = Y + 3.452Z - 3.908X \quad (3)$$

2.6.3 Pectin removal with ruthenium red staining

The residual pectin (%) on the fabric was evaluated with the ruthenium red $[(NH_3)_5RuORu(NH_3)_4ORu(NH_3)_5]Cl_6$ staining method [35]. Ruthenium red contains a hexavalent cation [36] which selectively binds to the intramolecular spaces of carboxyl groups of pectin [37]. The positively charged dye interacts with the negatively charged pectin carboxylic groups, leading to a dark reddish color after dyeing, and the pectin removal from the fabric can be judged from the differences in color intensity (K/S) of fabrics before and after scouring [35].

Fabrics (1 g) were dyed at 50 °C for 30 min in a dye solution containing 30 mg of ruthenium red per 100 ml of distilled water. The dyed samples were washed three times with distilled water at room temperature and air-dried for at least 24 hours before assessing K/S values at 540 nm [38] using a reflectance spectrophotometer (CM-3610D, Konica Minolta). Residual pectin content was estimated from a linear scale between K/S value from stained raw cotton fabric (considered 100 % pectin content) and dyed alkaline scoured fabric (0 % pectin residue).

2.6.4 Water absorption time or water drop test

The water drop test was performed according to NBR 13000, where the fabric was fixed in an embroidery frame and a burette containing distilled water (20 ± 2 °C) was positioned 40 mm from the fabric surface, with 5 s drop formation time. Water absorption time was defined as the time (in seconds) required for a water drop to be completely absorbed by the fabric, and it was calculated as the mean of 10 measurements made at different areas on the sample. The shorter the wetting time, the better the fabric absorbency. Absorption times longer than 180 seconds were not recorded, and the test was interrupted after 30 minutes.

2.6.5 Vertical wicking height

Vertical wicking height was monitored according to the standard test JIS L 1907:2004. The lower edge of a fabric sample (approximate size, 2.5 cm x 20 cm) was immersed (≤ 3 cm) vertically in a vessel with a solution of distilled water and dyestuff (1 g/l of direct blue dye). The wicking height of the cotton samples was measured after 10 minutes and the greater the height, the better the fabric vertical wicking.

2.6.6 Contact angle

To determine the hydrophilicity of the different treated fabrics water/fabric contact angle was also measured with a Ramé-Hart (Model 250) goniometer in static mode as described elsewhere [39].

2.6.7 Scanning electron microscopy (SEM)

The surface morphologies of the cotton samples were analyzed with a a JEOL JSM-6390LV SEM instrument at 10 kV and magnifications of 2500 and 5000 [39]. Prior to the observation, the samples were coated with a thin layer of gold.

2.6.8 Fourier-transform infrared spectroscopy (FTIR)

FTIR spectra of treated and untreated knitted cotton fabrics were obtained on a VERTEX 70 Fourier Transform Infrared Spectrometer with a Platinum-ATR-accessory, Diamond and OPUS Spectroscopy Software (Bruker, Germany) The spectra were collected through 64 scans over the range 4000-350 cm-1 at room temperature with a resolution of 4 μm. Data from FTIR spectra were treated in Origin 8.0. To reveal modifications in the region of 1730 cm-1 fabrics were treated for 5 minutes with concentrated HCl vapor. Therefore, fabrics were positioned above a beaker with concentrated HCl (~ 37 %). Vaporization with HCl results in protonation of carboxylic groups from pectin that are not visible without this procedure [40, 41].

2.6.9 Color coordinates and color strength (K/S)

Dyed samples were evaluated for the depth of the color by determining colorimetric coordinates in the CIELAB color space and the color strength (K/S), using a reflectance spectrophotometer (CM-3610D, Konica Minolta) at 640 nm. The K/S values were calculated according to the Kubelka-Munk equation (3), where K is the absorption coefficient of the sample, S is the scattering coefficient, and R is the absolute diffuse reflectance [17].

$$K/S = \frac{(1 - R)^2}{2R} \quad (3)$$

2.7 STATISTICAL ANALYSIS

All experiments and determinations were obtained from triplicate measurements, and results were expressed as mean ± SD (standard deviation). ANOVA analysis of variance, Tukey test and t-test were used to determine significant differences, with a significance level of 5 % (95 % confidence interval). Statistical analyses were performed using STATISTICA® software (version 7.1 and 12).

3. RESULTS AND DISCUSSION

3.1 EFFECT OF INCUBATION TIME ON ENZYME ACTIVITY

Enzyme stability depends not only on temperature and pH but also on the time that the biocatalyst is exposed to these conditions, which is especially important during industrial processes and when it is supposed to be reused several times. Furthermore, optimum pH and temperature are not necessarily the best conditions for prolonged use and reuse. Therefore, the stability of BioPrep® 3000L was evaluated by incubating it at 55 °C, pH 8.5 and in the presence of 1 g/l non-ionic surfactant (conditions that have previously been evaluated as optimal for pectin removal and wetting improved of fabrics) for 300 minutes, which corresponds to the total process time of 10 cycles of 30 minutes each treatment. Results are displayed in Figure 1.

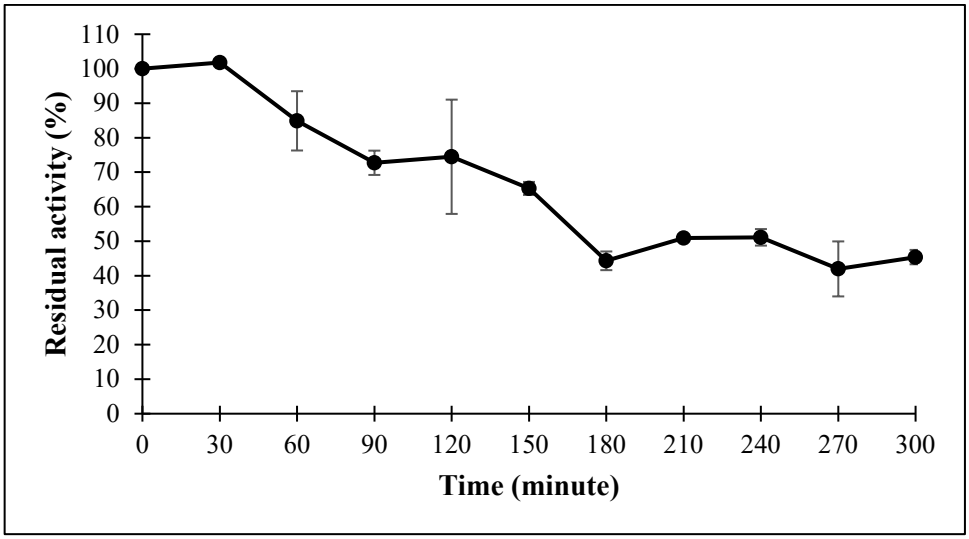


Figure 1 - Effect of incubation time on residual pectate lyase activity. The initial activity was set 100 %. Incubation experiments of pectinase BioPrep® 3000Lwere carried out at 55 °C and pH 8.5 for 300 min, in 50 mM Tris-HCl buffer and 1.0 g L^{-1} of non-ionic surfactant Berol® 175.

Results showed a continuous almost linear decrease of the initial activity of 28.61 ± 5.74 U in 100 mL or 0.2861 ± 0.057 U mL^{-1} within 180 minutes to 40-50 % of the initial activity and from 180 to 300 minutes enzyme activity remained nearly constant at 0.1332 ± 0.012 U ml^{-1}. The observed deactivation may be rather attributed to temperature effects on enzyme structure rather than surfactant interactions with the enzyme. Besides nonionic surfactants are reported to have little or no negative influence on most enzymes, while ionic surfactants might interfere negatively due to strong electrostatic interactions with ionic groups of proteins [42-44]. Lower pectinase activity can lead to a slower reaction rate and have a negative impact on the final quality of the scoured cotton fabric. However, according to Wang et al. (2007) [45], the complete elimination of pectins from the cotton fiber is unnecessary, because a previous investigation from Novozymes indicated that 22-30 % of residual pectin would not affect the water absorption of fabrics. Besides, the surfactant has proved to be an essential auxiliary to guarantee good wetting of the cotton fabric and because it has an important influence on the removal of waxes and fats [16, 17, 46] , and as a general rule nonionic surfactants can be regarded as more benign to an enzyme than anionic and cationic surfactants [43].

Note: Due to interferences of accompanying substances in the ultraviolet region of the spectrum it was not possible to measure the enzyme activity directly in the reused scouring baths.

3.2 EVALUATION OF BIOSCOURING CYCLES

To evaluate the reuse of pectate lyase (BioPrep® 3000L) containing bioscouring bath, the bioscouring process was carried out 10 times with the same bath, by adding only 11 ± 1 % of fresh enzyme solution (corresponding to 11 mL and 3.1 U) in each new cycle to compensate for liquor losses. Physicochemical properties of the 100 % cotton knitted fabrics were evaluated and results are shown in Table 1 and Table 2 and are compared to fabrics scoured by the conventional alkaline method with NaOH.

Table 1 – Physico-chemical properties of enzymatically treated (cycle 1-10) and alkaline scoured cotton fabrics.

Treatment		Weight Loss (%)	Whiteness (Berger)	Yellowness (ASTM D 1925)	Brightness (TAPPI 452/ISO 2470)	Residual Pectin (%)
Alkaline						
A 90ºC, 2g L^{-1}, 30 min		4.65 ± 0.09 [b]	48.50 ± 1.64 [a]	12.25 ± 0.971 [a]	70.68 ± 0.363 [b]	3.18 ±1.64 [a,b]
A 95ºC, 2g L^{-1}, 30 min		3.44 ±0.03 [d]	44.45 ±0,374 [b]	14.85 ±0.315 [b]	72.67 ±0.615 [a, b]	3.67 ±0.85 [a,b]
A 95ºC, 2g L^{-1}, 60 min		4.98 ±0.09 [a]	48.24 ±1.36 [a, b]	13.16 ±0.559 [b]	73.08 ±0.728 [a]	0.00 ±0.65 [c]
A 95ºC, 4g L^{-1}, 30 min		3.71 ±0.08 [c]	49.20 ±1.53 [a]	12.79 ±0.468 [b]	73.35 ±0.868 [a]	1.24 ±0.83 [a,c]
Bioscouring cycle	1	2.84 ± 0.14 [e]	35.969 ± 0.627 [c]	17.363 ± 0.305 [c]	66.988 ± 0.470 [c]	6.56 ± 0.42 [d]
	2	2.85 ± 0.07 [e]	34.603 ± 0.365 [c]	17.894 ± 0.092 [c]	66.455 ± 0.433 [c]	7.69 ± 0.80 [d, e]
	3	2.80 ± 0.05 [e]	34.319 ± 1.337 [c]	17.986 ± 0.461 [c]	66.248 ± 0.801 [c]	7.89 ± 2.74 [a, d, e]
	4	2.82 ± 0.10 [e]	34.511 ± 1.473 [c]	17.934 ± 0.475 [c]	66.398 ± 1.006 [c]	7.26 ± 0.21 [d, e]
	5	2.81 ± 0.05 [e]	33.216 ± 0.306 [c]	18.461 ± 0.018 [c]	65.991 ± 0.607 [c]	8.39 ± 1.65 [d, e]
	6	2.79 ± 0.08 [e]	33.192 ± 1.159 [c]	18.426 ± 0.420 [c]	65.797 ± 0.620 [c]	7.31 ± 0.81 [d, e]
	7	2.84 ± 0.02 [e]	33.360 ± 1.623 [c]	18.409 ± 0.571 [c]	66.058 ± 0.901 [c]	8.48 ± 1.03 [e]
	8	2.76 ± 0.03 [e]	33.206 ± 1.681 [c]	18.446 ± 0.557 [c]	65.908 ± 1.063 [c]	7.66 ± 0.30 [e]
	9	2.83 ± 0.07 [e]	33.311 ± 1.823 [c]	18.429 ± 0.711 [c]	66.032 ± 0.768 [c]	6.56 ± 2.19 [a, b, d, e]
	10	2.81 ± 0.02 [e]	32.395 ± 1.549 [c]	18.779 ± 0.508 [c]	65.625 ± 1.015 [c]	7.76 ± 1.51 [d, e]
Bioscouring Control		1.65 ± 0.04 [f]	32.637 ± 0.658 [c]	18.754 ± 0.314 [c]	65.957 ± 0.044 [c]	89.79 ± 9.47 [f]
Untreated (raw fabric)		0.00	16.340 ± 1.111 [d]	25.560 ± 0.391 [d]	58.130 ± 0.300 [d]	100.00 ±6.04 [f]

No pectinase was applied in the control. Values are the average from three replicates ± SD (standard deviation). Superscripts (a,b,…) are derived from Tukey tests (weight loss, whiteness, yellowness, brightness) or t-test (residual pectin) using Statistica 7.1. Equal letters in the columns of the same property mean that results are not statistically different at 95 % confidence interval.

Enzymatic scouring of cotton is accompanied by weight loss [19] as shown in Table 1, where all bioscouring cycles caused on average a weight loss of 2.81 %, which was significantly lower than that observed for alkaline scouring procedures. Between bioscouring cycles no significant differences in weight loss ($p>0.05$ by Tukey posthoc test and t-test) were found, which shows that non-cellulosic impurities of the cuticle and primary wall of the fiber are

equally removed, even with decreasing enzyme activity as expected from the stability test (see Figure 1). The highest weight losses (3.71-4.98 %) were obtained by alkaline scouring, which results from the severer process conditions and the removal of almost all fats, waxes, pectins, and proteins from the cotton fabric [18], but also some fiber damage such as cellulose depolymerization and fabric strength loss can be undesired side effects [47, 48]. Amongst alkaline scouring procedures carried out at 95 ºC, doubling of the process time from 30 to 60 minutes as well as doubling sodium hydroxide concentration resulted in slightly higher and statistically different weight loss. The lower weight loss obtained by enzymatic scouring shows in principle, that the non-cellulosic constituents were removed without significant cotton cellulose degradation [49]. Lower weight loss is an important commercial advantage since knitted goods are traded on a weight basis [50]. Weight loss decreased in the following order: alkaline scouring > bioscouring (cycle 1-10) > bioscouring control (buffer, surfactant).

The observed weight loss of the control treatment without enzyme (1.65 ± 0.04 %), can be attributed to water-extractable impurities [19] and the effect of the added surfactant [51]. It is interesting to note that the weight loss in fabric samples treated with pectate lyase is about 1.2 % higher when compared to treatment with buffer solution (control), which matches the maximum amount of pectin (1.2 %) in the cotton fiber, depending on the cotton supplier and the maturity of the fiber [52].

The degradation of pectins is a key step in the bioscouring process with enzymes, which facilitates the release of fatty and waxy substances [38]. The remaining pectin was stained with ruthenium red and results in Table 1 show that pectinase BioPrep® 3000L was equally efficient in the digestion of the available pectin during all ten bioscouring cycles, reducing pectin content by more than 91 %, and leaving in average only 7.56 ± 0.66 % of residual pectin on the fabrics, which is between 4 to 7.5 % more than on the alkaline scoured fabrics. According to the Tukey test (post-hoc, 95 % confidence interval) no significant statistical difference between residual

pectin content of cotton fabrics after each of the 10 cycles was verified (statistical test results not shown), but pectin content was significantly higher than in alkaline treated fabrics. Analyses with the t-test (see Table 1) confirmed differences between alkaline and bioscoured fabrics and revealed some additional, but marginal differences between different bioscouring cycles. Residual pectin content achieved by bioscouring cycle 1 was slightly lower than of other cycles and presented statistical difference to bioscouring cycles 7 and 8. Although t-test of residual pectin suggests that results from bioscouring cycles 3 and 9 are not statistically different to results from some alkaline scouring procedures, this can be attributed to the higher standard deviation of treatments 3 and 9, furthermore the p value was only slightly above 0.05. As expected, the control treatment had no significant effect on pectin removal and the treated fabric remained with high levels of pectic material (89.79 ± 9.47 %). Statistically the residual pectin content of the control treatment was equal to the raw fabric. It is remarkable that despite the decrease in enzyme activity observed in the stability test (see Figure 1) the efficiency of pectin removal was maintained during the 10 subsequent bioscouring cycles (Table 1). Bioscouring involves a solid substrate, where pectin composes the outer layers and requires the enzyme to adsorb on the fiber surface. Enzyme stability is complex because it can be influenced by various factors. It has been shown that the adsorption of enzymes to their substrates, to macromolecules or to other small molecules can increase their stability [53-55]. Analogous to this behavior it may be supposed that adsorption of pectinase or pectate lyase on pectic substances on the cotton fiber surface may increase enzyme stability and help to maintain the enzyme active and permit longer processing times. Furthermore, degradation or solubilization products released during bioscouring might aid to contribute somehow to enzyme stability. However, further investigations are necessary for elucidation.

The treatment solution became gradually yellower with reuse (Suppl Figure 3), indicating that some chromophoric substances were released to the treatment bath.

Consequently, the bioscouring process as a whole provided the removal of impurities from cotton and improved the whiteness index of the fabric by 98-120 % when compared to the raw fabric, increasing it from 16.34 ± 1.11 degree Berger to 33.81 ± 1.03 degree Berger. However, the whiteness of enzymatically scoured fabrics was not statistically different from the control treatment with buffer solution, which means that the addition of pectate lyase did not make any contribution to the fabric´s whiteness and the observed bleaching is mainly due to the washing effect and solubilization of colored impurities. Whiteness index, yellowness (18.21 ± 0.41), and brightness (66.15 ± 0.39) remained unchanged during the ten bioscouring cycles, without statistically significant differences (Tukey test at 5% probability) (Table 1). In contrast, alkaline scouring with NaOH, due to the more severe reaction conditions, achieved an almost fifteen units better whitening result (48.50 ± 1.64 degree Berger). Therefore, the enzyme process is more suitable for intermediate to dark-colored final products where the whiteness level is not highly relevant [22, 56]. For the complete removal of natural pigments responsible for the low white index of raw cotton, an additional bleaching stage is necessary [57].

Brightness of the treated fabrics behaved like fabric whiteness while for yellowness the opposite tendency was observed. While brightness increased for the control and alkaline treatments, yellowness decreased significantly for both treatments.

As reported above, about 91 % of pectin was digested, which is more than enough to destabilize the structure of the cuticle and primary cell wall of the cotton fiber and release non-cellulosic material by subsequent emulsification [50] to increase the fabric´s hydrophilicity [22]. Thus, all enzymatic treatments led to a huge improvement in water absorption of the fabrics (Table 2) when compared to the raw fabric, which did not absorb the water drop within 1800 seconds, when the measurement was interrupted. Fabrics from bioscouring cycles 1 and 2 displayed good hydrophilicity in terms of water absorption time, vertical wicking height, and contact angle. While vertical wicking height and contact angle were not found to be statistically

different from the alkaline treated fabric carried out at 90ºC with 2 gL^{-1} and for 30 minutes, differences in water drop absorption to all alkaline treated fabrics were evident. Alkaline treated fabrics absorbed the water drop instantaneously and absorption was found to be uniform at different points of the textile. Bioscoured fabrics showed nonuniform water drop absorption which resulted in high standard deviation of water drop absorption of these fabrics. The nonuniform absorption may be in part attributed to redeposition of hydrophobic matter previously removed from fabrics.

Table 2 – Water absorption time, vertical wicking height, and contact angle of untreated, enzymatically treated (reuse cycles 1-10) and alkaline scoured cotton fabrics.

Treatment		Water absorption time (s)	Vertical wicking height (mm)	Contact angle (θ)
Alkaline scouring				
A 90ºC, 2g L^{-1}, 30 min		< 1 s[a]	132.00 ± 1.73[a]	0.00 ± 0.00
A 95ºC, 2g L^{-1}, 30 min		< 1 s[a]	-	-
A 95ºC, 2g L^{-1}, 60 min		< 1 s[a]	-	-
A 95ºC, 4g L^{-1}, 30 min		< 1 s[a]	-	-
Bioscouring cycles	1	3.65 ± 1.94 [a, b]	131.33 ± 4.73 [a]	0.00 ± 0.00
	2	6.33 ± 0.76 [b, c]	117.67 ± 8.74 [a, b]	0.00 ± 0.00
	3	13.14 ± 2.45 [d]	105.33 ± 11.93 [b, c]	0.00 ± 0.00
	4	19.39 ± 3.84 [d]	105.67 ± 8.02 [b, c]	120.67 ± 0.96
	5	22.51 ± 7.40 [d]	96.33 ± 5.77 [b, c, d]	113.45 ± 4.69
	6	19.03 ± 9.58 [b, d]	92.67 ± 7.57 [c, d]	103.00 ± 9.39
	7	19.93 ± 8.42 [d]	92.00 ± 5.57 [c, d]	114.93 ± 2.52
	8	16.74 ± 6.94 [c, d]	89.00 ± 9.54 [c, d]	111.70 ± 7.39
	9	29.97 ± 16.05 [c, d]	77.33 ± 11.68 [d]	111.84 ± 0.32
	10	19.05 ± 4.90 [b]	91.33 ± 4.93 [c, d]	113.89 ± 7.79
Bioscouring control		> 90 s[e *]	91.67 ± 3.51 [c, d]	125.43 ± 4.10
Untreated (raw fabric)		> 1800[f #]	0.00 ± 0.00	134.24 ± 6.16

Values are the average from three replicates ± SD (standard deviation).
Values with the same superscript letters (a, b, c,….) derived from Tukey test (vertical wicking height) or t-test (water absorption time) are not statistically different within the 95% confidence interval.
[*] control fabrics displayed completely nonuniform behavior, but although some wetting was observed water drop absorption time was longer than 90 seconds.
[#] no water drop absorption was observed, but measurement was interrupted after 1800 s

From Table 2 it can be observed that water absorption time increases until the fourth bioscouring cycle and remains then more or less constant. Vertical wicking height showed similar behavior, but inversely proportional, it starts at 131 mm and decreases to 96 mm until the 5th bioscouring cycle and remains then in the range of 92 to 77 mm. The contact angle could

only be measured after the third bioscouring cycle and was considered equal to zero, as the water drop penetrated almost instantaneously, which is contrary to observations made by the water drop absorption test. For the fabrics of bioscouring cycles 4 to 10, θ oscillated between 120.67 ± 0.96 and 113.89 ± 7.79. Water absorption time, vertical wicking height and contact angle data, support the idea that fabric hydrophilicity remains constant after 4 reuse cycles. Despite the significant decrease in water absorption after 2 to 3 reuses of the bath, all fabrics presented sufficient hydrophilicity (<30 s) for industrial textile finishing, according to Abdulrachman et al. [9] and Kalantzi et al. [58]. High hydrophilicity and very short water absorption times are essential for processes with extremely short contact times between the textile and the finishing liquor such as in continuous dyeing (pad-dry, pad-steam, etc.) or printing, however batch dyeing in jet, overflow or laundry washing machines provides sufficient long immersion and contact time to guarantee uniform dyeing or finishing results.

Correlating pectin removal with water absorption time and wicking height provides evidence that pectin is not the only factor that influences fabric wetting. Despite the same degree of pectin removal, enzymatically scoured fabrics displayed quite different wettability (Figure 2). The observed worsening in water absorption may be attributed to the accumulation of soluble degradation products (of pectin) and impurities, removed from cotton fibers in previous cycles, in the scouring bath, that can impede the solubilization and removal of more impurities or even redeposit on the fiber surface. For Buschle-Diller et al. [59], a problem encountered in reusing the treatment bath is precisely the increasing contamination with continuous use and the redeposition of these contaminants on the cotton material. Surfactant concentration that was kept constant throughout the ten-bioscouring cycles, might have become the limiting factor to maintain the hydrophobic components such as fats and waxes emulsified and dispersed, and some substances might have redeposited on the fiber and fabric surface, reducing thus its hydrophilicity. Possibly a higher initial surfactant concentration or the addition of more

surfactant after the second or third reuse cycle would have avoided this negative side effect. However, as discussed above, the concentrating contaminants did not affect the efficiency of pectin removal, and apparently did not inhibit the commercial pectate lyase preparation.

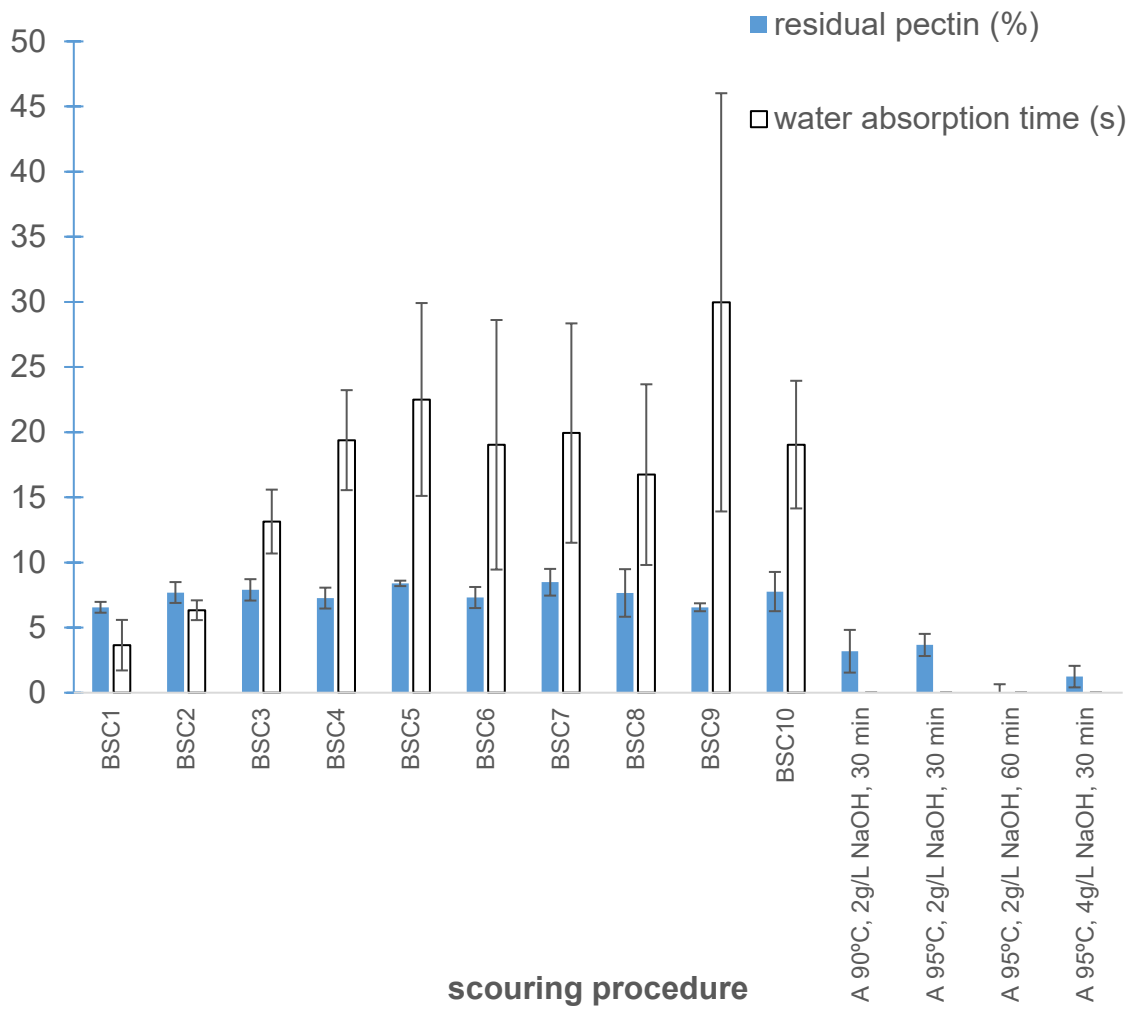
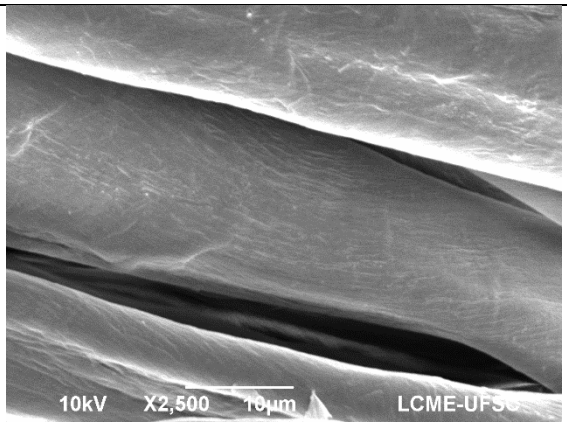
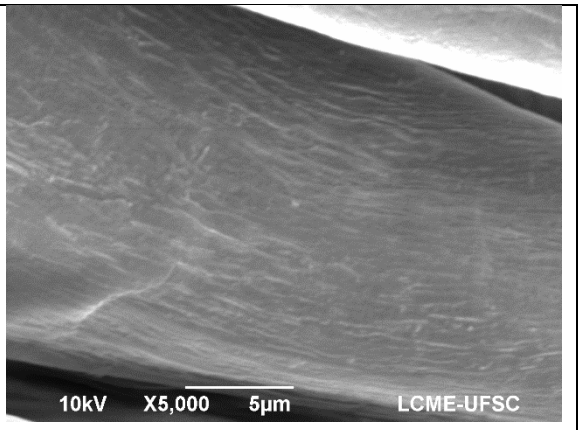
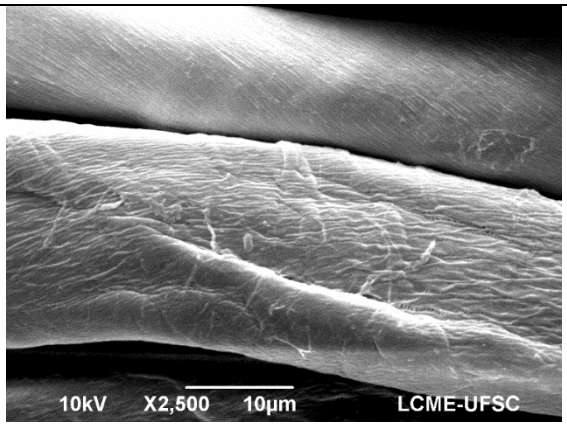
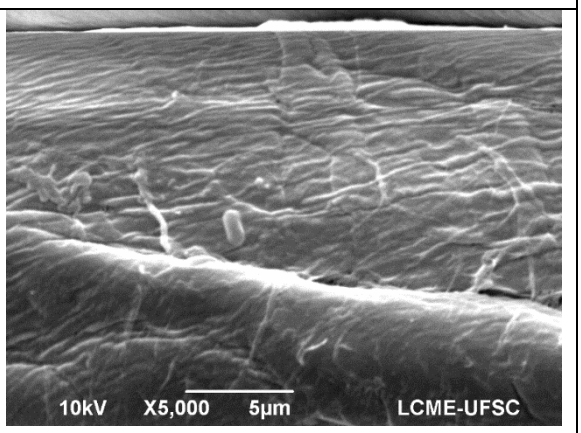


Figure 2 - Water absorption time (s) and residual pectin content (%) of cotton fabrics treated with BioPrep® 3000L with increasing bath reuse (bioscouring cycles BSC 1-10;) in comparison to alkaline scoured fabrics under different conditions (90-95ºC, 2-4 g L^{-1} NaOH, 30-60 minutes).

3.3 CHARACTERIZATION OF BIOSCOURED COTTON FIBERS

Figure 3 shows SEM images of cotton fabrics without treatment and after bioscouring cycles 1, 4, 7, and 10. It can be seen from the surface morphology that the secondary wall of the cotton fiber was exposed by bioscouring, in which the crystalline cellulose is visible in the

form of parallel and ordered microfibrils. In the sample from scouring cycle 1, fibrils and some other protruding structures appear on the fiber surface, which may be attributed to pectin and wax removal with partial destruction of the cuticle and primary cell wall. Li and Hardin [3] concluded that pectinases penetrate the cuticle through cracks or micropores, make contact with pectic substances in the substrate, and hydrolyze them, which results in the partial or complete breakdown and removal of the cuticle. Due to the specific and selective action of the pectate lyase, pectins are removed in the accessible areas and help to eliminate waxes, ashes, and other impurities that are intertwined and adhered to each other [11], revealing the main body of the cotton fiber. After the fourth use of the bioscouring bath, the surface of the fibers appears to be smoother. This may be a consequence of the decomposition products of pectin and waxes, which have been concentrating in the recycled scouring bath and redeposited as a thin film on the fiber surface, which would explain the increasing hydrophobicity of the fiber.

Raw		
1		

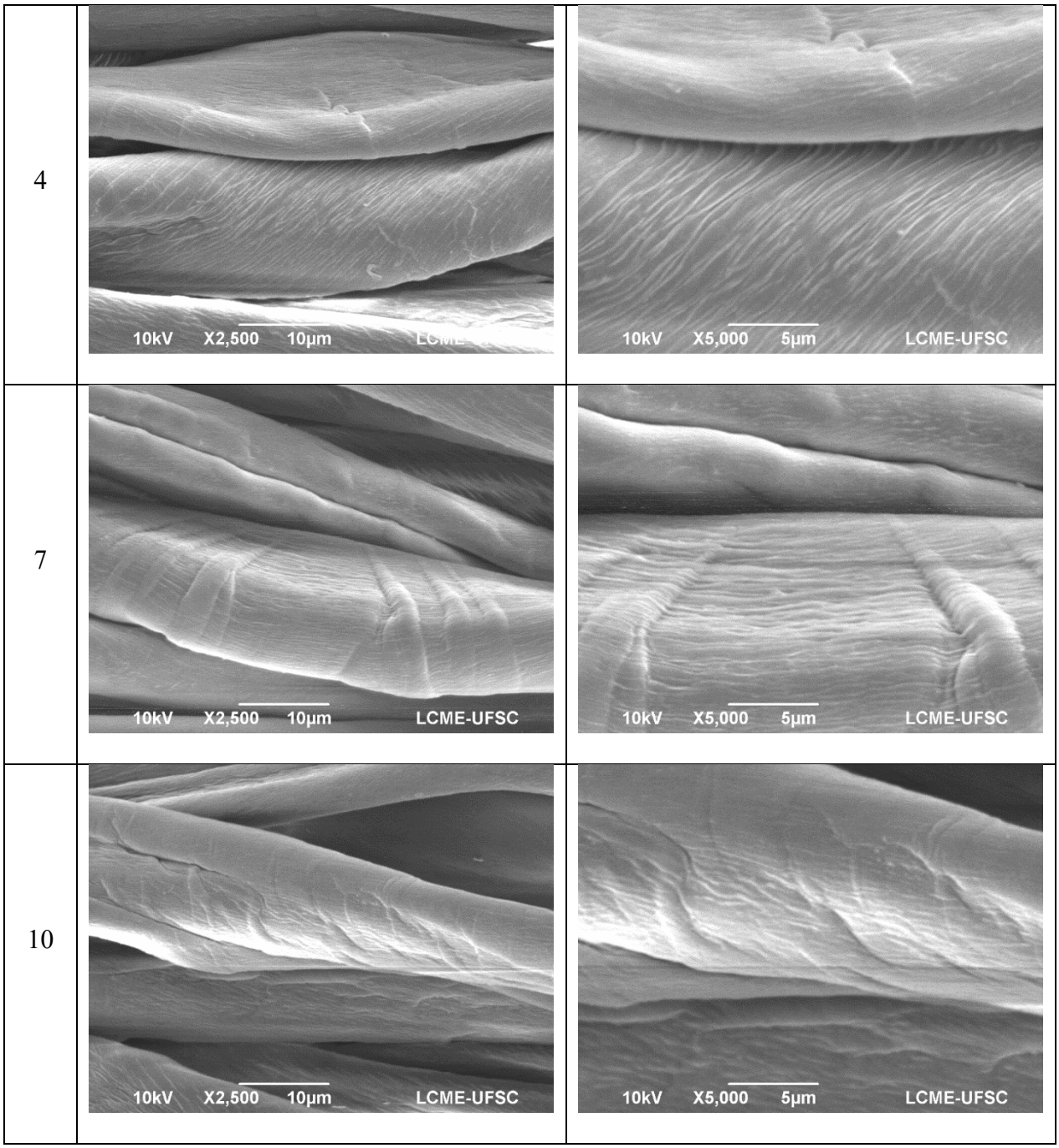


Figure 3 - SEM pictures of the surface of raw cotton fabrics and after treatments with pectinase enzyme, from bioscouring cycles 1, 4, 7, and 10, respectively (x 2.500 and x 5.000).

Infrared spectroscopy is an analytic technique rather used to identify substances because of chemical differences in bulk properties and its potential to identify impurities in small quantities is limited. The main changes expected in FTIR spectra of cotton after scouring are related to pectic substances and hydrophobic fats and waxes. However, infrared vibration bands of these substances are not easily visible and mostly covered by or buried in typical cellulose

bands or bands of adsorbed water. A detailed list of the bands encountered in cotton and cellulosic fibers can be found in Chung et al. [40] and [60, 61].

Carboxylate ions of pectate appear as bands around 1600 cm^{-1} (asymmetric stretching of $-COO^-$) and 1400 cm^{-1} (symmetric stretching of $-COO^-$), but they are hardly visible. To better visualize differences, all fabrics were exposed for 5 minutes to vapors of concentrated HCl before FTIR spectra were taken. As previously described, HCl vapors protonate the ionized carboxylate groups ($-COO^-$) encountered in pectins, transforming these ionized groups with two identical -C-O bonds (bond order 1.5) in carboxylic acids (-COOH) with a double bond (-C=O) and a single bond -C-OH. Therefrom a new peak is made visible in the range of 1720-1750 cm^{-1}, characteristic for carbonyl (C=O) stretching vibration. A second band for -C-OH stretching at 1200 cm-1 remains hidden amongst other C-O bands in this region [40, 41]. As it can be seen from Figure 4, basically no difference can be seen between the spectrum of the raw cotton fabric and the alkaline treated fabric when the fabric was not treated with HCl vapor. However, after HCl protonation an additional peak around 1720-1730 appeared for the raw fabric, and the region on the right side (~1600-1620 cm^{-1}) of the 1645 cm^{-1} band of adsorbed water diminishes slightly in intensity, which means that the carboxylate ions were protonated and shifted to the bands of -C=O and -C-OH. These additional bands and changes cannot be observed in the spectrum of the alkaline scoured fabric, which is a clear indication for the removal of the carboxylic groups in pectin.

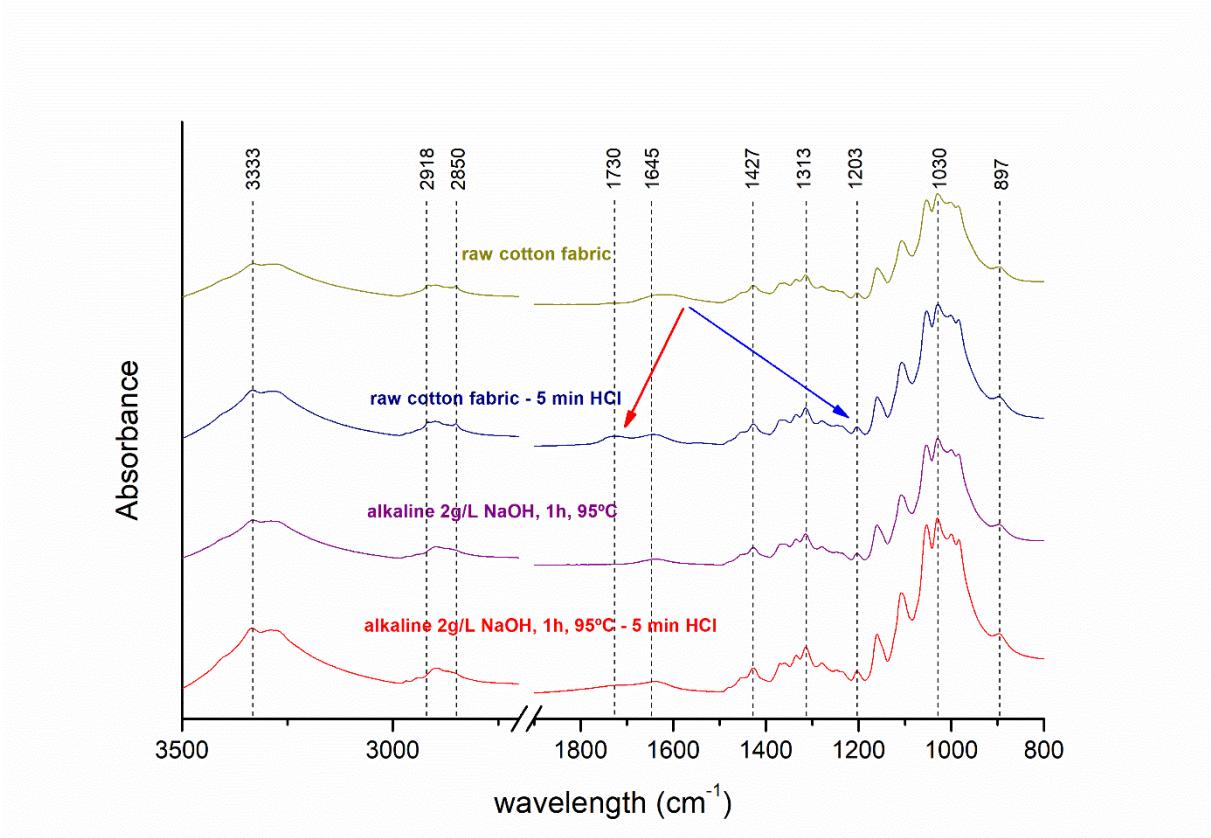


Figure 4 - FTIR spectra of raw and alkaline treated (2g L-1, 95 ºC, 60 minutes) knitted cotton fabrics without and with exposure to vapor of concentrated HCl solution.

As alkaline scouring is used as reference treatment for the evaluation of bioscoured fabrics, slightly different alkaline scouring conditions with respect to sodium hydroxide concentration (2 and 4 g L^{-1}), temperature (90 and 95 ºC) and scouring time (30 and 60 minutes) have been evaluated. The FTIR spectra for these different conditions are shown in Figure 5. The characteristic peak at 1730 cm-1 for -C=O stretching can be seen clearly only in the spectrum of the raw cotton fabric, while in all alkaline scoured fabrics this peak is not visible, which confirms the removal of pectin. The spectrum of the greige fabric shows two other additional bands around 2850 and 2918 cm^{-1} that cannot be detected in the alkaline scoured fabrics.

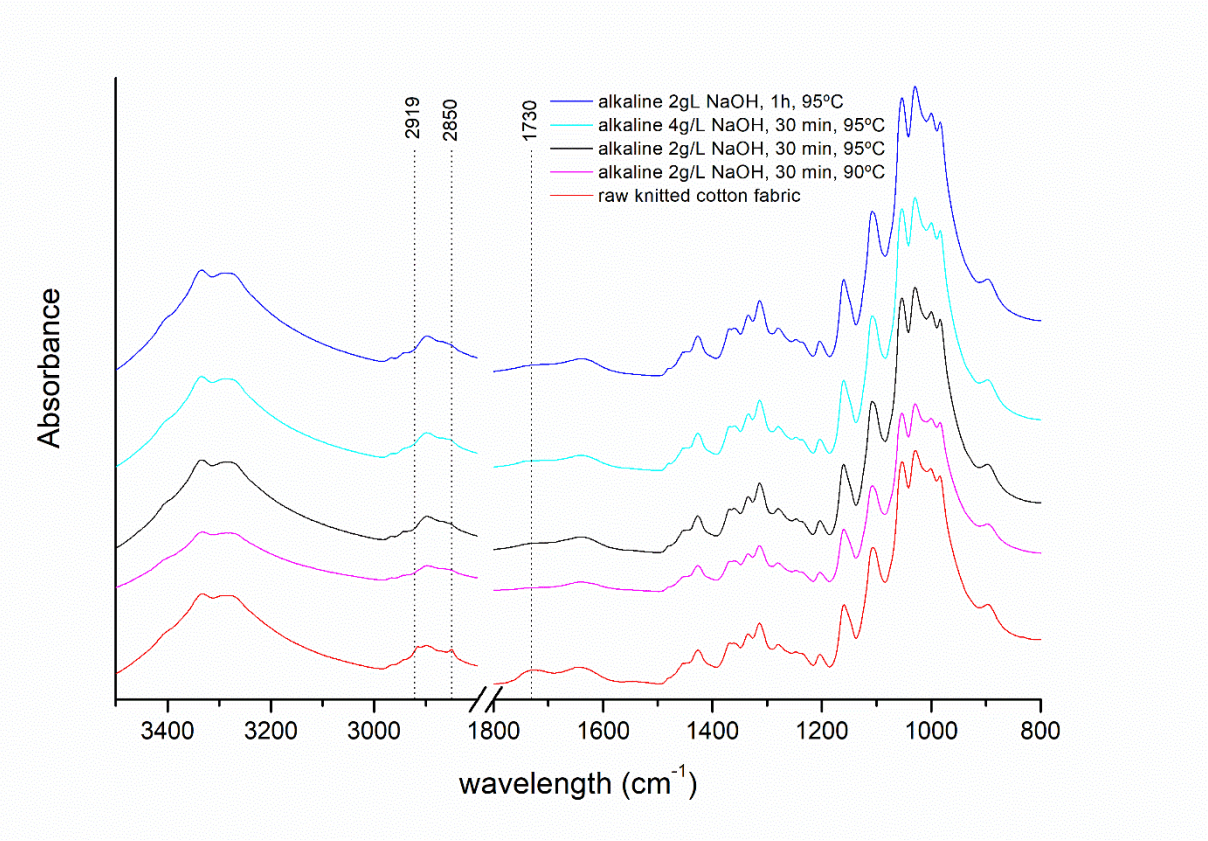


Figure 5 - FTIR spectra of raw knitted cotton fabric and cotton fabrics scoured under different alkaline conditions. All fabrics were treated with concentrated HCl vapor for at least 5 minutes before analysis.

These peaks are attributed to symmetric and asymmetric stretching of methylene (-CH_2-) groups of long alkyl chains [40] present in the hydrophobic fatty and waxy compounds present in the cuticle and primary cell wall of cotton, such as long chain alcohols, paraffins, esters and triglycerides, long chain carboxylic acids. The same difference between raw and alkaline scoured fabrics is also visible in Figure 4 in the spectra obtained from fabrics without HCl vaporization. Above 2900 cm^{-1} we observe that the spectra of the alkaline treated fabric lose intensity while the spectra of raw cotton fabric maintain approximately the same intensity until 2918 cm^{-1}. These spectral changes are proof for the modifications in the cotton substrate caused by alkaline scouring and the removal of hydrophobic substances containing long chained -CH_2- moieties. In the regions between 800 – 1600 cm^{-1} and above 3000 cm^{-1} no differences between raw and alkaline scoured fabrics were detected. Furthermore, no significant differences between

the FTIR spectra of the various alkaline scouring procedures could be detected. This is in good agreement with the results obtained for water drop absorption, however different residual pectin contents were estimated for the 4 different alkaline scouring procedures (Table 1). Despite of the good quality of the obtained FTIR spectra the pectin related peaks are of too low intensity for quantification of the differences between scoured fabrics.

FTIR spectra obtained for the fabrics from different bioscouring cycles (1, 4, 7 and 10) are presented in Figure 6 and are compared to the untreated raw cotton fabric, a control fabric treated under bioscouring conditions but without enzyme and alkaline scoured fabrics ($2gL^{-1}$ NaOH, 30 min at 90 ºC or 60 minutes at 95 ºC).

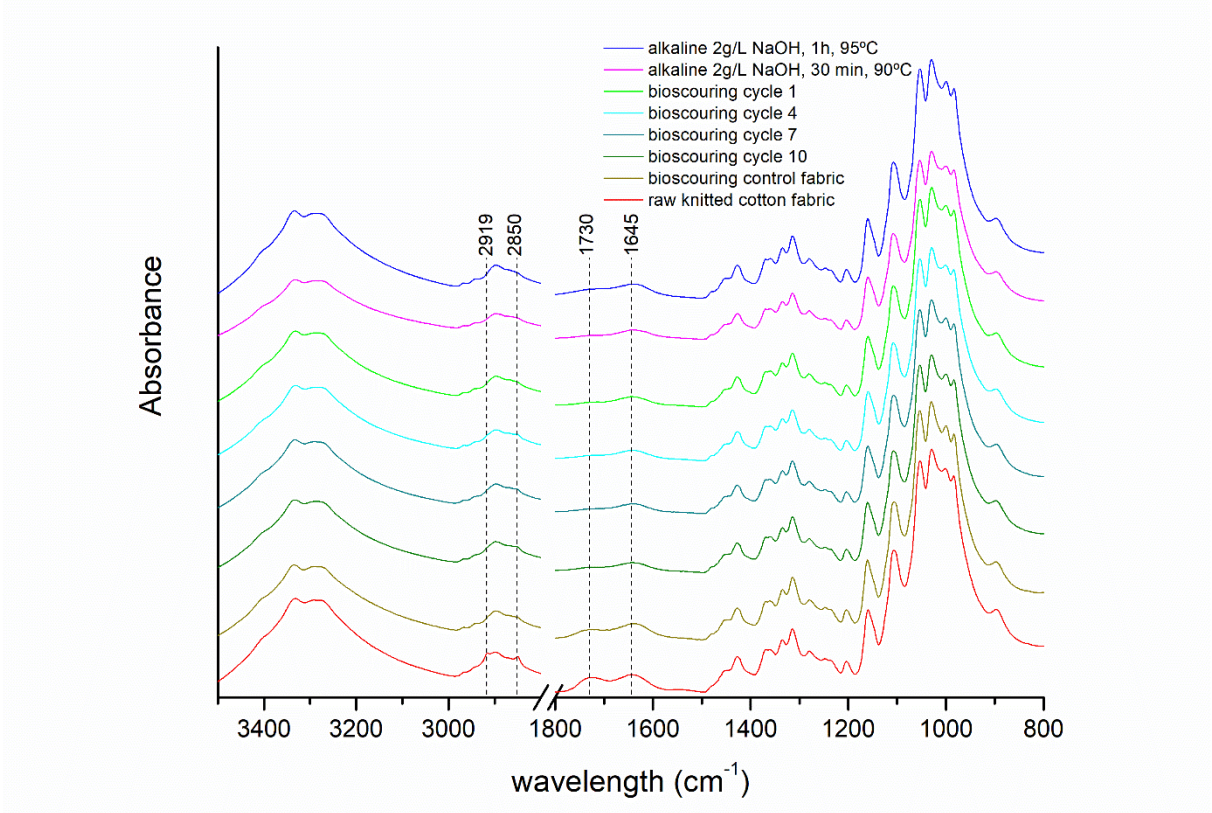


Figure 6 - FTIR spectra of raw knitted cotton fabric, control fabric for bioscouring, alkaline scoured fabrics and fabrics from bioscouring cycle 1, 4 7 and 10. All fabrics were treated with concentrated HCl vapor for at least 5 minutes before analysis. For better comparison spectra were normalized before plotting.

All spectra are very similar in the ranges from 800 – 1600 cm^{-1}, from 1900 – 2800 cm^{-1} and above 3000 cm^{-1}, but differences between raw, control and (bio)scoured fabrics can be

observed, as discussed above, for the peak intensities around 1600-1650, 1730, 2850 and 2918 cm^{-1}. Spectra of the bioscoured fabrics are practically indistinguishable from the alkaline scoured fabrics, suggesting that similar cleaning efficiencies are achieved by both treatments. Around 1730 cm^{-1} only the bioscouring control fabric shows a similar characteristic band for -C=O stretching as the raw cotton fabric, which means that carboxylic groups from pectin are still present. However, peak intensity is lower than for the untreated cotton, suggesting partial pectin removal by the washing process. At 2850 and 2918 cm^{-1} differences are less pronounced, especially between the control fabric and the other treated fabrics, but a visible difference exists between the raw fabric and the control fabric, indicating that the washing process without enzyme or alkali removes some hydrophobic impurities. Furthermore, no clear differences between spectral FTIR results of different bioscouring cycles are apparent, which suggests that similar results after the reuse of the scouring bath are obtained. For more details see also Suppl Figure 4. The obtained spectra are very similar because during scouring the drop in the concentration of impurities and of weight loss is too small (2.81 % weight loss on average for bioscouring and around 4 % for alkaline scouring) to drastically modify the FTIR spectra, indicating that no significant changes in the cellulose structure have occurred.

3.4 EVALUATION OF THE COLORATION PROCESS

Dyeability and dyeing quality of cotton depend on the surface and structural properties of the fibers, which are greatly affected by processes preceding textile dyeing [20]. Inadequate preparation can cause non-uniform dyeing problems that only become apparent after coloration. For this reason, careful pre-treatment is very important to ensure high and uniform dye absorbency by the substrate [8]. Table 3 shows the dyeing results (color coordinates, color strength, and color difference) of the fabrics treated with pectinase reuse (baths 1, 4, 7, and 10) in comparison to the reference fabric treated with alkali.

Table 3 – Color parameters of alkaline and bioscoured fabrics after dyeing with Reactive Blue 222 (1.7 % o.w.f.).

Treatment		K/S (640 nm)	L*	a* (-)	b* (-)	C*	h*	ΔE
Alkaline[+]		4.91 ± 0.45 [a]	46.74 ± 0.90	4.97 ± 0.48	25.02 ± 1.12	25.51 ± 1.01	258.67 ± 1.51	---
Reuse cycles	1	5.32 ± 0.08 [ab]	45.81 ± 0.21	4.96 ± 0.32	25.55 ± 0.60	26.03 ± 0.53	258.97 ± 0.93	0.75 ± 0.30
	4	5.25 ± 0.15 [ab]	45.95 ± 0.41	5.02 ± 0.39	25.45 ± 0.83	25.94 ± 0.74	258.80 ± 1.18	0.72 ± 0.30
	7	5.56 ± 0.14 [b]	45.26 ± 0.28	4.89 ± 0.33	25.77 ± 0.66	26.23 ± 0.59	259.22 ± 0.96	0.90 ± 0.54
	10	5.71 ± 0.06 [b]	45.01 ± 0.01	4.77 ± 0.29	26.15 ± 0.73	26.59 ± 0.67	259.61 ± 0.91	1.11 ± 0.73

Values are the average from three replicates ± SD (standard deviation).
[a, b] Values with the same superscript letters (a, b, etc.) derived from Tukey test are not statistically different at 5 %.
[+] The alkaline treated fabric used as reference for dyeing was scoured at 90 ºC with 2 gL-1 NaOH for 30 minutes.

Color strength (K/S) of the chemical and biological treated fabrics varied in a narrow range, with color differences ΔE (in comparison to the alkaline scoured fabric) lower than one. Considering that, as established by the clothing industry, ΔE has to be less than or equal to one, the obtained dyeing results show that each of the enzymatic scouring cycles could replace alkaline scouring as pretreatment for subsequent dyeing, printing, and finishing operations. The visual appearance was consistent with the spectrophotometrically measured results and all samples were uniformly dyed, with homogeneous color, and free from any defects, which can be attributed to good hydrophilicity of the fabrics and the high degree of pectin removal (>91 %), because the hydrophobic characteristic of pectic substances prevents dye uptake and causes stains [62].

Bioscoured fabrics showed satisfactory coloring with a small decrease in L* luminosity and a slight improvement in color strength (K/S), compared to alkaline treated fabric. Nerurkar and co-workers [21] also observed improved color yield for reactive dyes when the fabric was enzymatically scoured. A deeper color of the bioscoured fabric can result from the more yellow color of the bioscoured fabric or, another possible reason is that the remaining alkaline residues

on the alkaline scoured fabrics may cause hydrolysis of reactive dyes and reduce color strength [14].

The different pretreated fabrics exhibited similar dye exhaustion from the dyeing bath (Figure 7), with a characteristic decay of the reactive dye concentration versus time for dyeings with a chemical reaction, which can be attributed to the quite similar hydrophilicity and the efficient pectin removal of the fabrics. It is possible to clearly distinguish the two fundamental physicochemical phenomena that are typical to reactive dyeing: (1) substantivity (< 30 minutes), where the dye build-up on the fibers occurs due to adsorption, diffusion, and migration of the dye molecules; and (2) fixation (> 30 minutes), where the dye molecules react with the fiber due to the addition of alkali.

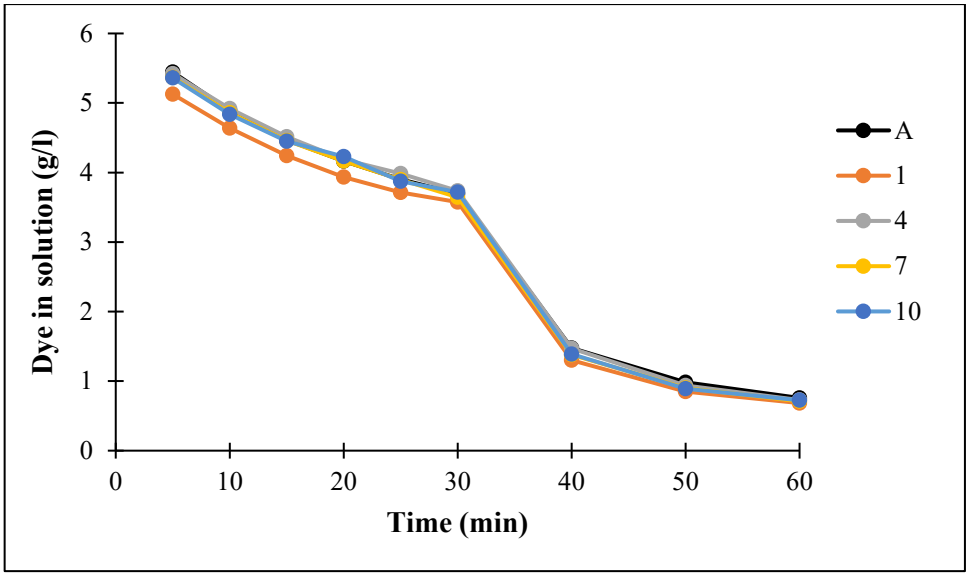


Figure 7 - Dyeing exhaustion curve for dyeing of cotton fabrics enzymatically treated with bath reuse (1, 4, 7, and 10) and conventionally scoured (A = alkaline) with C.I. Reactive Blue 222.

In summary, the comparison of water absorption (see Table 2) and color measurements of the dyed fabrics (see Table 3), treated with pectinase and alkali, indicates a slightly better water absorption of the chemically pretreated fabric but no appreciable differences in the K/S values after dyeing with Reactive Blue 222. As already pointed out above very fast water drop absorption (below 1 second) and excellent wetting are essential for fast finishing processes with short contact times or hydrophilic textiles, but good to medium wetting times are sufficient for

batch dyeing. The long contact time (30 minutes or more) between textile and dyeing bath and the alkaline dyeing conditions compensate for lower hydrophilicity.

4. CONCLUSIONS

Operating under carefully controlled conditions, the same bioscouring bath containing a commercial pectate lyase could be successfully applied in ten consecutive scouring cycles with crude knitted cotton fabric, delivering fabrics of high quality. The enzyme scoured cotton fabrics, regardless of the reuse cycle, retained more weight than the corresponding fabric subjected to traditional alkaline scouring. All bioscoured fabrics offered acceptable but not excellent water absorption characteristics, promoting satisfactory dyeing behaviors and color yields. Only 7.5 % of residual pectin was found in the ruthenium red test. The whiteness degree, however, is unsatisfactory and an additional bleaching would be necessary for dyeing of light colors. But although, the whiteness index of chemically treated substrates was higher than the enzymatically treated, color strength (K/S) was similar after dyeing with 1,7 % o.w.f. of reactive dye. The increase of soluble impurities in the reused bioscouring baths did not impair (inhibit) the enzyme performance and did not pose any problem to the process and the final product, however redeposition of accumulating impurities in the scouring bath on the fabrics may be responsible for the reduced hydrophilicity after the 3 scouring cycle and further investigations are necessary.

Ten bioscouring cycles could be carried out with one initial bioscouring bath complemented with only 11 % of the fresh bioscouring solution, allowing for considerable savings of enzyme, surfactant, and water, which means lower production costs and less effluent being discharged to the environment. The investigated approach results in a substantial reduction of consumables, capable to promote economic and environmental improvements, simultaneously, which is in line with the need for technologies that permit the reuse of process

water. The ten bioscouring cycles are thus a simple, economical, and comprehensive solution that can make the reuse of process water a reality on an industrial scale.

The reuse of the bioscouring bath makes the enzyme-based process more competitive and meets the needs of the modern textile industry for greener and more sustainable processing.

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Declarations Conflict of interest: The authors declare that they have no conflict of interest.

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5. SUPPLEMENTARY MATERIAL

A SUSTAINABLE APPROACH FOR COTTON BIOSCOURING: REUSE OF THE PECTATE LYASE CONTAINING TREATMENT BATH

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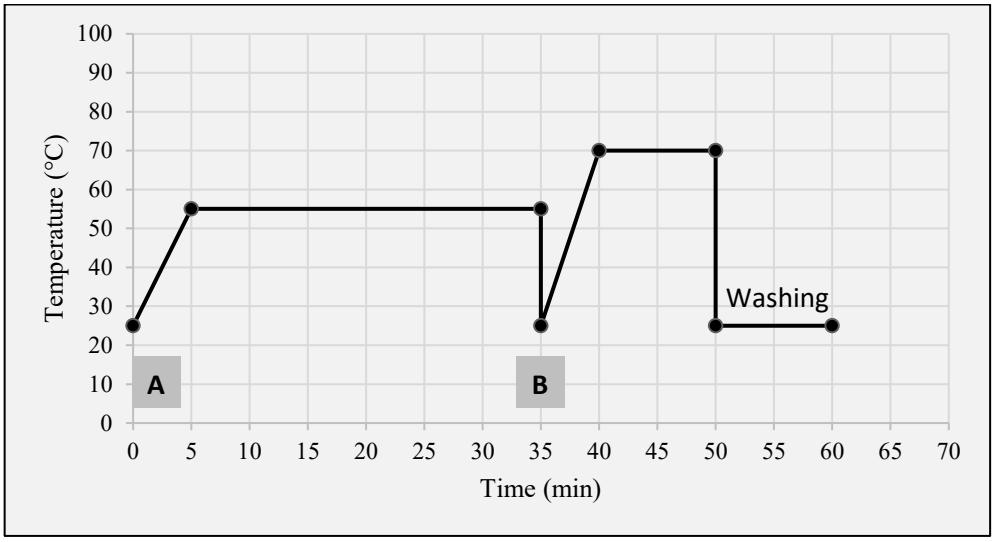
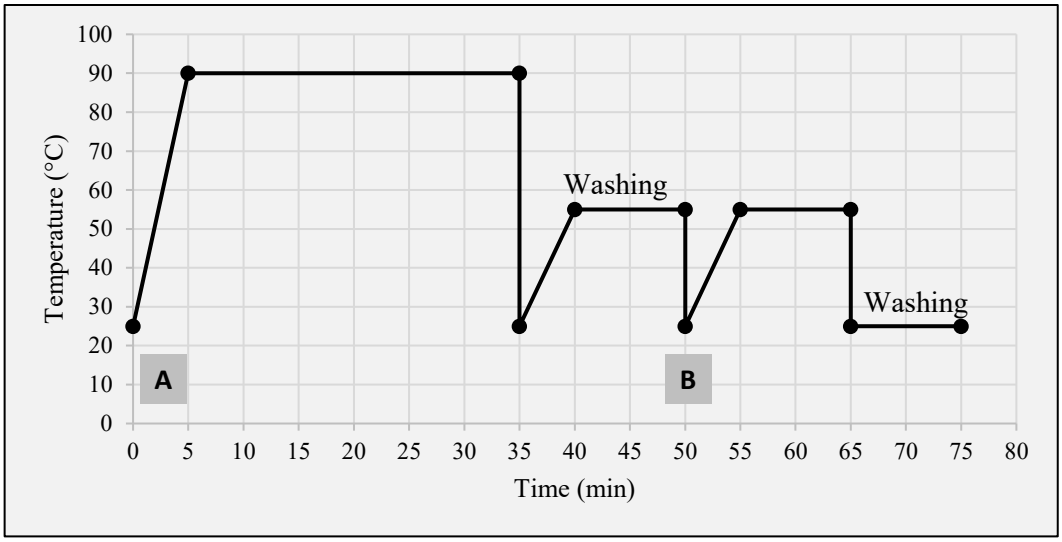
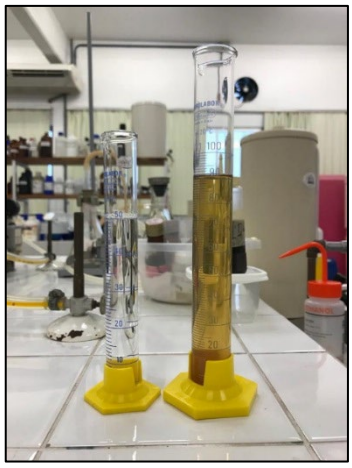


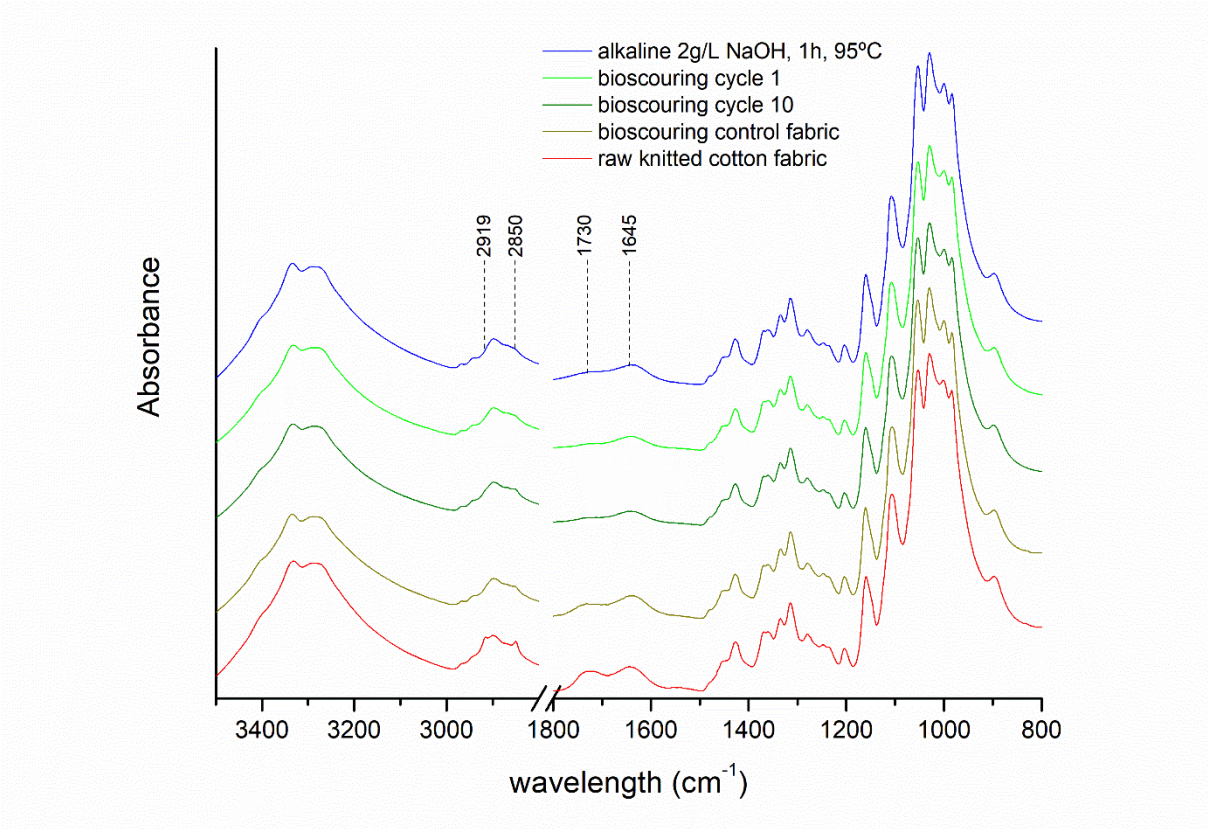
Suppl Figure 1 - Process scheme for enzymatic scouring of cotton knits. Solution A: BioPrep® 3000L (1.0 g L^{-1}) and non-ionic surfactant (1.0 g L^{-1}), in pH 8.5 buffer. Solution B: non-ionic surfactant (0.2 g L^{-1}).



Suppl Figure 2 - Process profile for alkaline scouring of cotton knits. Solution A: NaOH (2.0 g L^{-1} or 4g L^{-1}) and non-ionic surfactant (1.0 g L^{-1}). Solution B: acetic acid (3.0 g L^{-1}). Scouring temperature was varied 90 or 95ºC and scouring time was 30 or 60 minutes.



Suppl Figure 3 - Solution pigmentation of bioscouring in baths 1 (initial) and 10 (final), respectively.



Suppl Figure 4 - FTIR spectra of raw, alkaline treated (2g L^{-1}, 95 ºC, 60 minutes), bioscoured (cycle 1 and 10) and control fabrics. without and with exposure to vapor of concentrated HCl solution. All fabrics were treated with concentrated HCl vapor for at least 5 minutes before analysis. For better comparison spectra were normalized before plotting.