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Note

Characterization of bioscoured cotton fabrics using FT-IR ATR spectroscopy and microscopy techniques

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Abstract—The effects of bioscouring were investigated by characterizing the chemical and physical surface changes of cotton fabrics using a purified pectinase enzyme from *Bacillus subtilis* strain WSHB04-02. Fourier-transform infrared (FT-IR) attenuated total-reflectance (ATR) spectroscopy, scanning electron microscopy (SEM), and atomic force microscopy (AFM) techniques were employed. FT-IR ATR spectroscopy provided a fast and semi-quantitative assessment of the removal of pectins and/or waxes on the cotton surface by comparing the changes in intensity of the carbonyl peak induced by HCl vapor treatment at around 1736 cm^{-1}. The bioscoured surface could be clearly distinguished from those of untreated and alkali-treated cotton fibers using a combination of SEM and AFM. The images produced using these techniques revealed that the surface morphography and topography of the cotton fibers were shaped by the etching action mode of pectinases during bioscouring. These findings demonstrated that AFM is a useful supplement to SEM in characterizing cotton surfaces.

Keywords: Atomic force microscopy; Bioscouring; Characterization; Cotton; FT-IR attenuated total-reflectance spectroscopy; Scanning electron microscopy

Greige cotton (i.e., untreated or raw cotton) contains various non-cellulosic impurities present in the cuticle and primary cell wall of the fiber. The non-cellulosics include pectins (0.4–1.2%), waxes (0.4–1.2%), proteins (1.0–1.9%), ashes (0.7–1.6%), and other miscellaneous compounds.[1] Pectins as found in greige cotton mainly consist of neutral and acidic heteropolysaccharides with different molecular weights and degrees of esterification.[2] Waxes consist of fatty alcohols, fatty acids and their esters, and other hydrocarbons.[3] It is now generally accepted that the pectin and wax contents, and the distribution of the latter, are responsible for the non-wetting behavior of greige cotton by water. Bioscouring refers to the enzymatic removal of pectins and waxes from the surface of a cotton fiber, which endows it with improved bleachability and dyeability. This process preserves the fiber's structure and strength, and avoids the high energy consumption and severe pollution problems that are associated with conventional alkaline treatments.

Much of the work in the area of cotton bioscouring has been focused on investigating the utility of various enzymes. Although several types of enzyme—including pectinases,[4–9] cellulases,[2,4,8] proteases,[1,8] cutinases,[10] xylanases,[2,11] and lipases[2,12]—have been studied, pectinases have proved to be the most effective and suitable for cotton bioscouring. The mechanism of pectinase scouring reportedly assumes that the degradation and elimination of pectins makes the loosened waxes more easily removable with the help of surfactants and mechanical agitation; this allows the cotton to achieve superior hydrophilicity without fiber deterioration. It is clear that quantifying and imaging chemical and

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physical changes of bioscoured cotton surface are important to understanding the bioscouring mechanism in greater detail. However, neither staining tests (such as Ruthenium Red and Methylene Blue for pectins,[2,4,5] and Oil Red O for waxes[13]) nor weight-loss measurements[5,9] are precise quantitative methods. Moreover, although extraction methods (such as oxalate extraction for pectins[14] and organic solvent extraction for waxes[4,5]) can accurately determine the pectins and waxes contents, the processes involved are more laborious and time-consuming than other approaches.

Fourier-transform infrared (FT-IR) attenuated total reflectance (ATR) spectroscopy could highlight changes in the main non-cellulosic impurities by characterizing the carboxyl acids and esters that are present in pectins and waxes, which do not exist in the cellulose structure.[15] Scanning electron microscopy (SEM) was used to observe the surface characteristics of bioscoured cotton fibers in several previous reports.[2,4,5] Nevertheless, this technique cannot provide informations on height and roughness of the sample surface, which are important for understanding the enzymatic action on substrates located on the outer surface of cotton fibers. By contrast, atomic force microscopy (AFM) can generate fine surface topographies of samples at atomic resolutions and may be a useful supplement to SEM in characterizing cotton surfaces. However, so far, there have been no reports on the characterization of the surface of bioscoured cotton using this technique. In fact, relatively little work has been done to investigate the surfaces of any enzyme-treated fibers using AFM.[16]

The aim of the current study was to characterize the chemical and physical surface changes of cotton fabrics scoured with pectinase, and to identify appropriate methods for evaluating the effects of bioscouring on cotton fabrics. We therefore investigated the surface characteristics of bioscoured cotton treated with pectinase PL from *Bacillus subtilis* strain WSHB04-02, and compared them with those of greige cotton and alkali-scoured cotton using FT-IR ATR spectroscopy, SEM, and AFM.

FT-IR ATR spectra of greige cotton and HCl-treated cotton (Fig. 1) showed characteristic cellulose peaks around 1000–1200 cm^{-1}.[17] Other characteristic bands related to the chemical structure of cellulose were the hydrogen-bonded OH stretching at ca. 3550–3100 cm^{-1}, the CH stretching at 2917 cm^{-1}, the asymmetrical COO^- stretching at 1617 cm^{-1}, and the CH wagging at 1316 cm^{-1}.[15] It should be noted that the identification of the carbonate ion band around 1700–1600 cm^{-1} by FT-IR ATR is quite difficult because the OH bending of absorbed water (1642 cm^{-1}) was also observed in this regions. Figure 1 showed a rather weak absorbance at around 1736 cm^{-1} in the FT-IR ATR spectrum of greige cotton, which might be attributable to the presence of the carboxylic ester in pectins and waxes. According to Chung,[15] when cotton fabrics were exposed to HCl vapor for a few minutes, the ionized carboxylate (COO^-) was converted into carboxyl (COOH) through protonation; accordingly, a characteristic peak of carbonyl (C=O) caused by stretching vibration appeared at around 1750 cm^{-1} in the FT-IR ATR spectrum. This was confirmed by the spectrum of bioscoured cotton shown in Figure 1. The enhanced absorption at around 1736 cm^{-1} should be attributed to the protonation of the ionized carboxylate because the ester groups in pectin and/or wax of greige cotton fabric merely presented a very weak absorption. Whether or not these ester groups could be hydrolyzed into carboxyl groups, they might not be the main contribution to the enhancement of the absorbance at around 1736 cm^{-1}.

As shown in Figure 2, in the current study, the absorbance intensity of the characteristic peaks at around

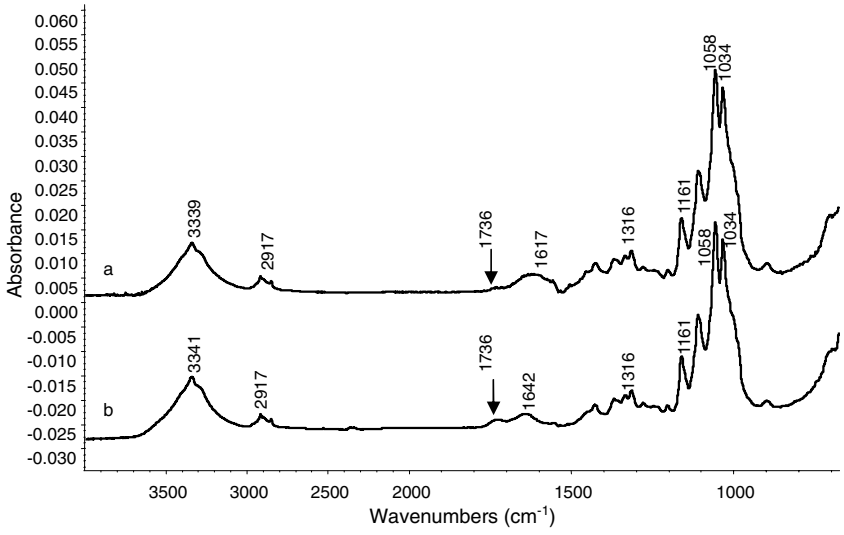

Figure 1. FT-IR ATR spectra of (a) greige cotton and (b) greige cotton treated with HCl vapor.

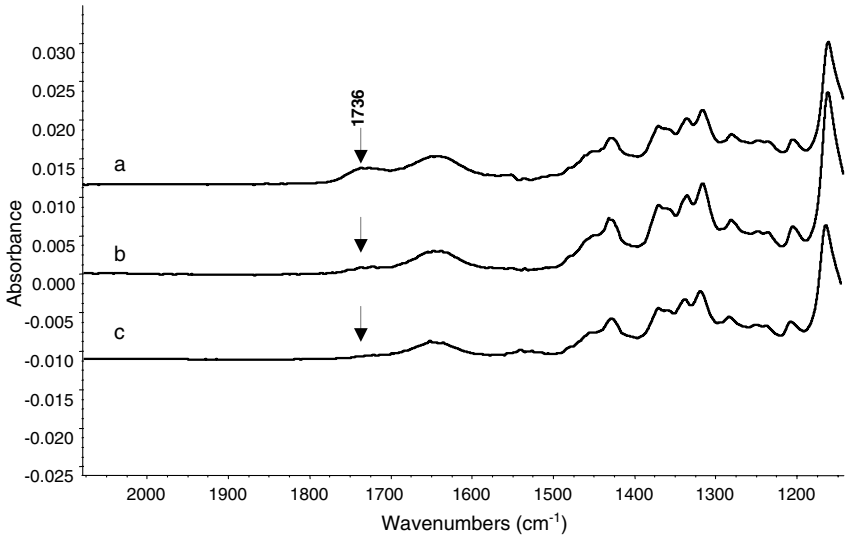

Figure 2. FT-IR ATR spectra of (a) greige cotton, (b) bioscoured cotton, and (c) alkali-scoured cotton treated with HCl vapor.

1736 cm^{-1} varied in the following order: greige cotton > bioscoured cotton > alkali-scoured cotton. The characteristic peak almost disappeared in the FT-IR ATR spectrum of the alkali-scoured cotton fabric. These results indicated that although the enzymatic scouring of cotton fabrics could partially eliminate non-cellulosic impurities (pectins and/or waxes), the process was not as effective as alkaline scouring. This could be attributed to different scouring mechanisms of the pectinase and alkali, which are further revealed by SEM (Fig. 3) and AFM (Fig. 4) analyses. However, the bioscouring process allowed the fast and semi-quantitative assessment of the removal of the main impurities on the cotton surface by comparing the changes of absorption intensity at around 1736 cm^{-1}.

The SEM image of the surface of greige cotton (Fig. 3a) revealed characteristic parallel ridges and grooves, as reported previously by Li and Hardin.[4] The entire surface was covered with primary wall structure. The bioscoured samples showed unevenly distributed cavities or concave grooves (Fig. 3b). These novel features can be attributed to the selective etching action of the pectinase on pectins in the primary wall. The specificity and accessibility to the substrates (pectins) of the pectinase should be responsible for this kind of action mode. As shown in Figure 3c, the surface features of the alkali-scoured cotton were clearly different from those of the bioscoured cotton. The parallel ridges and grooves had almost vanished in the bioscoured and alkali-scoured cotton, whereas the latter presented a flatter and smoother surface without pronounced cavities. This indicated that the alkali had a peeling effect on the non-cellulosic impurities.

A topographical examination by AFM revealed changes in the surface characteristics of the enzyme-treated and alkali-treated cottons (Fig. 4). The greige cotton presented a relatively rough surface, with a maximum height of 350.00 nm and an average height of 224.0 nm (Fig. 4a). Pectinase and alkali treatments lowered the maximum heights to 334.71 and 198.02 nm, with average heights of 222.0 and 96.1 nm, respectively (Fig. 4b and c). The unevenly distributed cavities or concave grooves visible in the SEM image of the bioscoured cotton showed a clear depth distribution (represented by the color gradation described on the right-hand side of the AFM image). It should be noted that the vertical stripes on the right-hand side of Figure 4b might be artifacts of the AFM. Roughness analysis of the AFM images (Table 1) showed that the greige cotton had the greatest surface roughness, followed by the bioscoured cotton and the alkali-treated cotton. The SEM and AFM images combined with the FT-IT ATR analysis supported the conclusion that alkaline scouring removed more non-cellulosic impurities from the cotton surface than enzymatic scouring.

1. Experimental

1.1. Materials

Purified alkaline pectinase (lyase) PL, which was isolated from strains of *B. subtilis* WSHB04-02 in the Key Laboratory of Industrial Biotechnology (Ministry of Education, Southern Yangtze University, Wuxi, China), was used for cotton bioscouring in the current study. The enzyme was stored at 4 °C before the experiments. Greige cotton knits (14.5 tex, 150 g/m^2) were supplied by Wuxi Knitting Factory (China). All other chemicals were of analytical grade unless indicated.

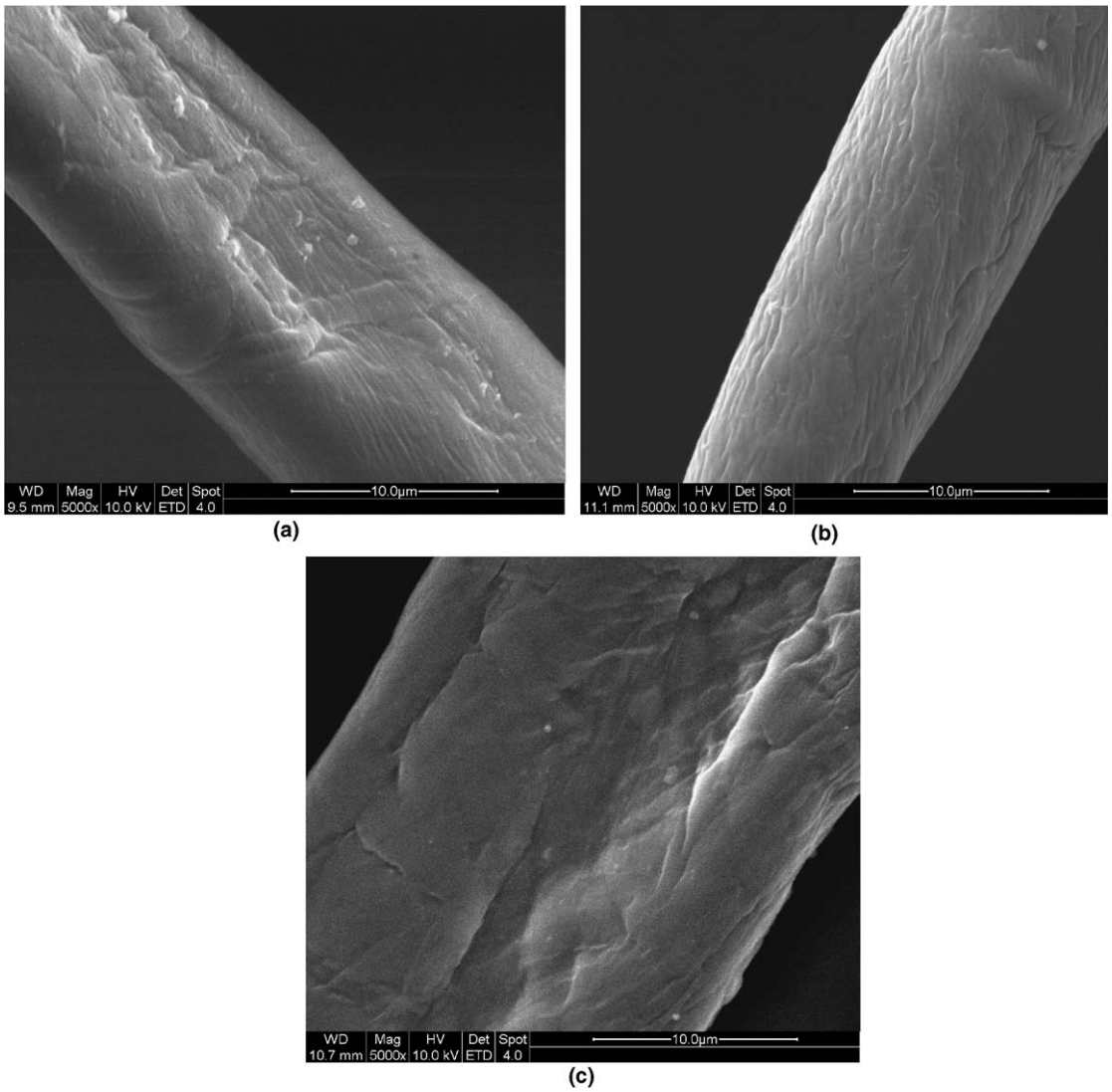

Figure 3. SEM images of (a) greige cotton, (b) bioscoured cotton, and (c) alkali-scoured cotton.

1.2. Scouring experiments

Cotton knitted samples (10 g) were incubated in 1.0 g/L pectinase solution buffered with 0.05 M glycine/NaOH buffer solution (pH 9.1) at 57 °C for 75 min with a liquor-to-fabric ratio (v/w) of 20:1. All of the experiments were carried out in a WHYF-2F thermostatic bath (Shanghai Yuejin Co., Ltd., China) at a moderate stirring speed (level 5 from levels 0–10) in the presence of 0.1% (w/v) non-ionic surfactant (TX-10), which acted as a wetting agent. After enzymatic treatment, the samples were washed with boiling water for 2 min to deactivate the enzymes, followed by washing with distilled water for three cycles. The treated cotton knits were finally air dried.

Conventional scouring of greige cotton knits was carried out in a WHYF-2F thermostatic bath at a moderate stirring speed (level 5) as follows. Cotton knitted samples (10 g) were incubated in a solution of 2 g/L NaOH and 0.5 g/L TX-10 with a liquid-to-fabric ratio of 20:1 at 98–100 °C for 90 min, followed by rinsing and air drying as described above. All of the scouring experiments were duplicated.

1.3. Surface characterization of bioscoured cotton

1.3.1. FT-IR ATR. A Thermo Nicolet Nexus FT-IR spectrophotometer (Thermo Electron Corporation, MA, USA) equipped with an OMNI-Sampler, a DTGS detector, and a Ge-on-KBr beamsplitter (7800–350 cm^{-1}) was used for the FT-IR ATR measurements to indicate the changes in the main characteristic groups of non-cellulosic impurities on the cotton surface. The FT-IR ATR spectra (64 scans, 4 cm^{-1} resolution) were recorded using a single reflection horizontal ATR accessory with a spherical Ge crystal fixed at an incident angle of 45°. A sample with a 2-mm diameter was measured.

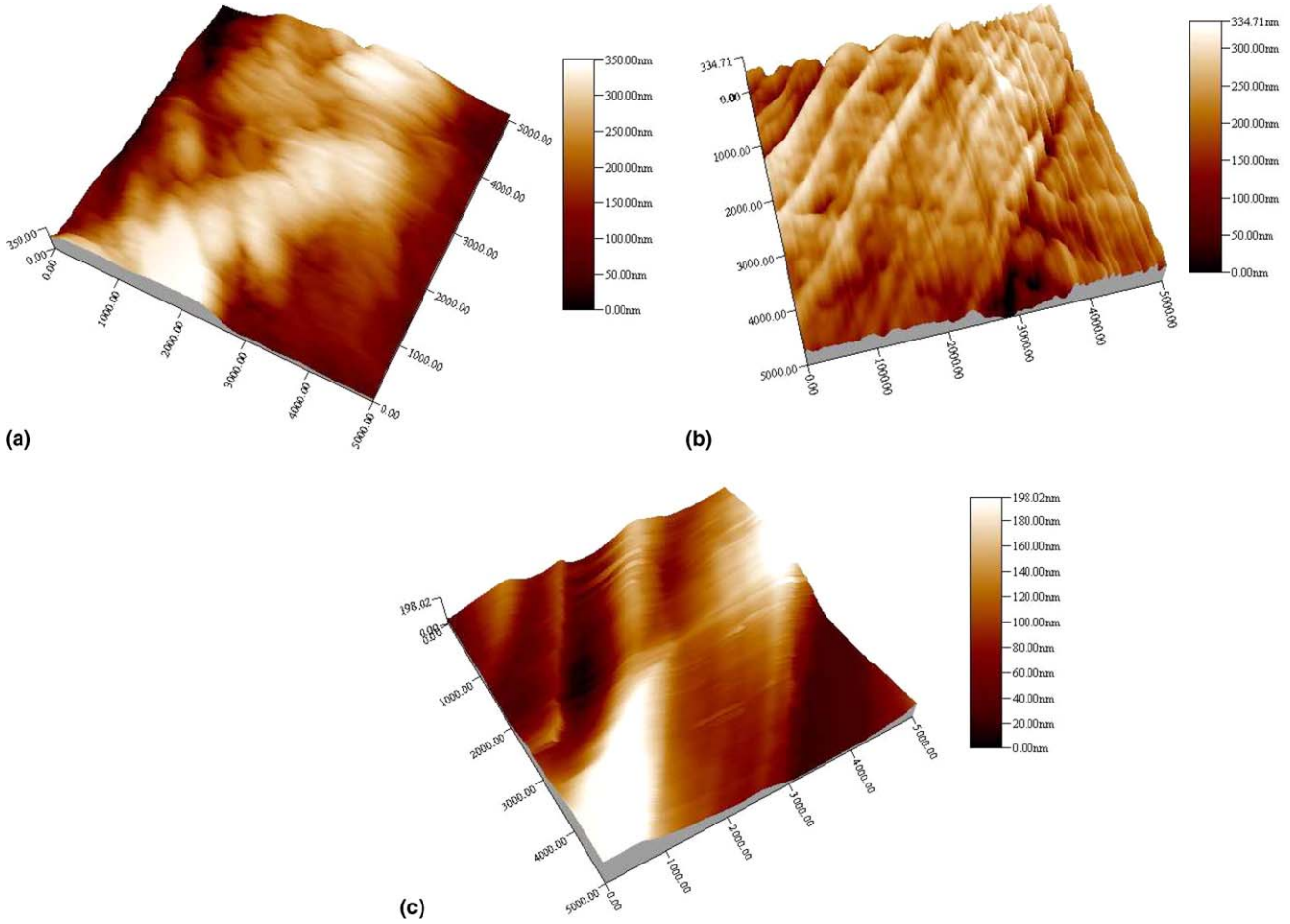

Figure 4. AFM images of (a) greige cotton, (b) bioscoured cotton, and (c) alkali-scoured cotton.

Table 1. Surface roughness analysis

Samples	S_a [a]	S_q [b]	S_k [c]	S_{vk} [d]
Greige cotton	75.1	87.2	214	97.3
Bioscoured cotton	41.2	53.4	123	80.2
Alkali-scoured cotton	40.8	51.8	111	57.8

[a] S_a: Average roughness, nm.
[b] S_q: Root means square roughness, nm.
[c] S_k: Core roughness depth, nm.
[d] S_{vk}: Reduced valley depth, nm.

1.3.2. SEM. The surface morphologies of the cotton samples were visualized using a FEI Quanta-200 scanning electron microscope (FEI Company, The Netherlands), operating at a typical accelerating voltage of 10 kV. The samples were sputter-coated with gold for 40 s at 15 mA prior to the observation.

1.3.3. AFM. A commercial multimode sample-scanning AFM (model CSPM-3300, Ben Yuan Ltd., China) was used for imaging the surface of the cotton specimens. The vertical resolution of the apparatus was 0.1 nm, and the horizontal resolution was 0.2 nm. All of the AFM images were obtained in the contact mode with a silicon nitride cantilever at a scanning speed of 1.0 Hz and a scanning range setting of 5000×5000 nm under ambient conditions.

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