Changes in Dyeability and Morphology of Cotton Fiber Subjected to Cellulase Treatment

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ABSTRACT: Unprocessed and mercerized cotton fibers were treated with commercial crude cellulase. The changes in the dyeability and structural features of the fiber due to cellulase treatment were studied. The dyeability was examined in terms of uptake of three reactive dyes and the apparent affinity of Congo Red to cotton fiber. The dyeability of the unprocessed fiber was assumed to be influenced by some impurities present in it. This fiber probably resembled polynosic fiber in molecular aggregate at a certain stage of hydrolysis. Mercerized cotton showed a similar pattern in dyeability as weight loss increased, regardless of dye species. Enzyme more easily penetrated the mercerized fiber than the unprocessed fiber. Cellulase treatment influenced the X-ray crystalline reflection pattern for the mercerized fiber but nominally influenced that for unprocessed fiber. Scanning electron micrographs revealed that cellulase treatment caused swelling of the fibrils. They also revealed that the disordered regions between the fibrils in the secondary walls were removed at low weight loss for the unprocessed fiber. The mercerized fiber at high weight loss had large cracks oblique to the fiber axis and showed no individual fibrils in the secondary wall. The primary wall was removed in the initial stage of hydrolysis for both the unprocessed and mercerized fibers. © 1997 John Wiley & Sons, Inc. J Appl Polym Sci **65:** 155–164, 1997

Key words: cellulase; cotton fiber; mercerization; dyeability; structural features

INTRODUCTION

Cellulase has been used for finishing cellulose fibers such as cotton fiber. The dyeability of hydrolyzed cotton fiber differs markedly from that of regenerated fibers.[1] One reason for this is the highly ordered structure composed of fibrils and cell walls in natural fiber.[2]

There exist many reports concerning the influence of highly ordered structure on the enzymolysis of cotton. Walseth[3] suggested that the secondary wall is more difficult to hydrolyze with cellulase than the primary wall. Focher et al.[4] showed that enzyme penetration occurs more easily in cotton fiber than in regenerated cellulose fiber due to the larger pore size of the former.

Cotton fabric is a comfortable and readily available material for human clothing. Therefore, studying the cellulase treatment with respect to the structure and morphology of cotton is important. Moreover, studying the dyeing of fabrics is not only relevant in relation to the production of clothing, but also provides insight into the microstructure of fibers. The relationship between the structure and dyeability of slack mercerized cotton fiber is examined here, compared with that of unprocessed cotton fiber. Slack mercerization is a practical method for achieving such properties as elasticity of cotton fibers.[5]

In a previous article,[6] cellulase-treated cupra

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and polynosic fiber were dyed with three reactive dyes. Polynosic fiber showed markedly different dye uptake depending on dye species. This was assumed to be due to changes in the molecular aggregate caused by cellulase treatment. In contrast, dye uptake in cellulase-treated cupra was far less influenced by dye species.

In this study, we first used four dyes: three reactive dyes and Congo Red. On the basis of the results, we selected two reactive dyes, which showed entirely contradictory behavior in terms of molecular aggregate for cellulase-treated polynosic fiber,[6] in order to examine dyeability of the mercerized cotton fiber.

These results were studied in relation to structure and morphology of the fiber examined using X-ray, infrared, and scanning electron microscope measurements.

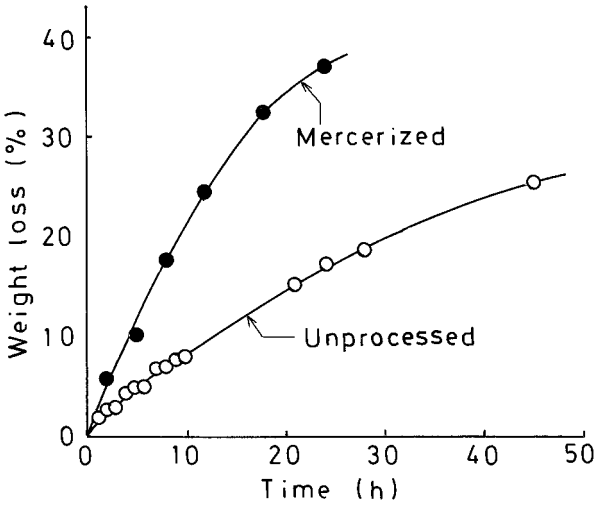

Figure 1 Weight loss caused by cellulase treatment versus treatment time for the unprocessed and mercerized fibers.

EXPERIMENTAL

Sample

The unprocessed cotton fabric used (JIS L 0803 shirting fabric; U fiber) was the same as that described in previous articles.[1,6,7] Prior to experimentation, the fabric was treated using an aqueous solution of 0.1% nonionic surfactant.

Reagent-grade Congo Red was used. The reactive dyes used were commercially available dyes, C.I. Reactive Blue 19 (Blue 19), and two recently developed dyes[8] (dye A and dye B). Blue 19 is a monofunctional vinylsulfone dye. Both dye A and dye B have a chromophore bonded to the vinylsulfone group via a monochlorotriazine group. The vinylsulfone group is more active than the monochlorotriazine group in dye A and dye B. Therefore, the three reactive dyes have almost the same functionality.[6] These dyes were described in detail in a previous article[6] and used without further purification.

Mercerization Method

Fiber structure is thought to depend on mercerization conditions, such as temperature and alkali concentration. Slack mercerization was performed in the conventional manner.[9] Unprocessed cotton was immersed in an aqueous solution of 20% NaOH at room temperature for 3 min. After this treatment, the cotton fabric was thoroughly rinsed with pure water, followed by rinsing with 0.1*N* aqueous acetic acid solution, then washed again with pure water at room temperature, and finally dried. These mercerizing conditions were taken from the literature.[10]

The weight loss versus enzyme treatment time plots are shown in Figure 1. The hydrolyzing velocity of mercerized fiber (M fiber) was much higher than that of U fiber. We obtained weight losses of 17.3 and 37.1% after 24 h for U and M fibers, respectively. The enzyme-treated U and M fibers are called UE and ME fibers hereafter, respectively.

Method of Enzyme Treatment

The fiber was treated under the conditions of a liquor-to-sample ratio of 1 : 100, 0.2% enzyme concentration, pH 4.5, and temperature of 40°C. After a given treatment time, enzyme was inactivated in boiling water, then the fiber was washed with 0.1% aqueous ammonia, and finally rinsed thoroughly with pure water.

Dyeing Method

Fiber was dyed to equilibrium with Congo Red at 80°C. Other dyeing conditions were described previously.[7] Dyeing with dye A, dye B, and Blue 19 was performed under the conditions described previously.[6] Apparent affinity (affinity) of Congo Red to cotton fiber was calculated using the equation described in a previous article.[7] The effective volume term (volume) for dyeing of U fiber was assumed to be 0.22 L/kg, as used previously.[7] Dye

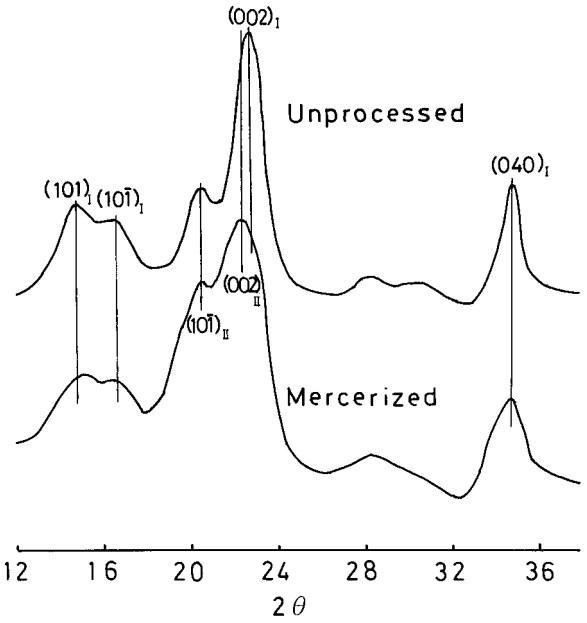

Figure 2 XRD patterns of the unprocessed and mercerized cotton fibers. Subscripts I and II of planes indicate cellulose I and cellulose II, respectively.

uptake was obtained in the same manner as described previously.[6]

X-ray Measurement

An X-ray diffractometer (SRA M18XHF; MAC Science Co.) was used to obtain the crystallinity X_c, which was in the 2θ range of 12 to 38 degrees in the present study. The other conditions used to obtain X_c were the same as described previously.[6]

X-ray diffraction (XRD) patterns of U and M fibers are shown in Figure 2. U fiber had crystalline reflections from (002), (101), (10$\bar{1}$), and (040) planes of cellulose I.[11] There was also a small peak of reflection near the (002) plane. This is the reflection from the (10$\bar{1}$) plane of cellulose II.[12,13] Therefore, we infer that U fiber consists of celluloses I and II.[14]

The (002) reflection in M fiber shifts to a smaller 2θ than that in U fiber. This reflection was attributed to the presence of cellulose II crystallites.[12,13] The (10$\bar{1}$) reflection of cellulose II in M fiber was much stronger than that in U fiber. These results indicate extensive phase transition from cellulose I to cellulose II upon mercerization. However, since the reflections from (101), (10$\bar{1}$), and (040) planes of cellulose I were still observed in M fiber, it was assumed that M fiber contains a small amount of cellulose I.

Lewin and Roddan[15] reported that slack mercerization decreased the order of crystallites. Line broadening of the independent (040) reflection for M fiber seems to be greater than that for U fiber. This coincides with results described in the literature.[15]

Infrared Spectroscopic Measurement

A spectrophotometer (FTS-30; BIO-RAD Co.) was used to obtain the crystallinity index I_c, which was defined in the same manner as described previously.[6] Diffuse reflectance infrared Fourier transform (DRIFT) was adopted as a sampling method.[16]

Raman Spectroscopic Measurement

A spectrophotometer (FT-Raman; BIO-RAD Co.) was used to check the cellulose crystallite type[14] in the Raman shift range of 200 to 3500 cm^{-1}.

Observation by Electron Microscope

Scanning electron microscopes (S-2400 and S-2460N; Hitachi Co.) were used. The samples were coated with gold. All micrographs were taken at accelerating voltage in the range of 10 to 15 kV.

RESULTS AND DISCUSSION

Dyeability of Cellulase-Treated Unprocessed Cotton Fiber

Uptake of Blue 19, dye A, and dye B by U fiber is shown in Figure 3. The affinity of Congo Red to U fiber, which is 8.33 kcal/mol, as reported previously,[7] is also shown in Figure 3. The affinity was calculated using a constant volume value of 0.22 L/kg, regardless of weight loss. Accordingly, the changes in the affinity with weight loss were assumed to be based on the change in volume caused by the cellulase treatment.[1,7] Congo Red showed a minimum affinity at about 10% weight loss, then the affinity gradually increased with increasing weight loss. This showed that the molecular structure was relaxed by a C_1 enzyme. The number of regions dyeable with Congo Red increased after this relaxation when weight loss exceeded about 10%. Volume of UE fiber was calculated by numerical calculation, provided that 8.33 kcal/mol is the affinity of Congo Red to cellulose.

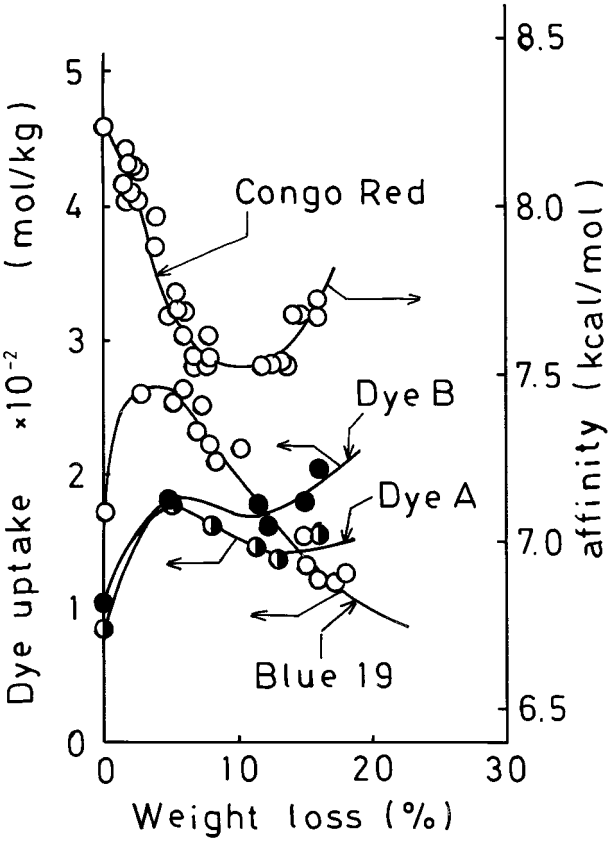

Figure 3 Uptake of three reactive dyes and apparent affinity of Congo Red versus weight loss caused by cellulase treatment of the unprocessed fiber.

The volume ratio of the smallest value of 0.17 L/kg in UE fiber to 0.22 L/kg in U fiber was 0.77.

As previously reported,[6] Blue 19 and dye B showed entirely contradictory behavior in the dye uptake versus weight loss plots for polynosic fiber.[6] Also, dye A showed a behavior between those of Blue 19 and dye B.[6] In this study, uptake of Blue 19, dye A, and dye B rapidly increased up to a weight loss of about 5%. Then a decrease was observed, followed by a gradual increase with weight loss greater than about 10% for dyes A and B. In contrast, uptake of Blue 19 decreased monotonically with increasing weight loss when weight loss exceeded 5%.

The minimum uptake and affinity value appeared at around 10% weight loss for dye A, dye B, and Congo Red. The difference in dye uptake between 20% weight loss and 10% weight loss was greater for dye B than for dye A. Also, the affinity of Congo Red was higher at 20% weight loss than at 10% weight loss in a similar manner as uptake of dye B. Since uptake of Blue 19 continues to decrease with increasing weight loss, the changes in dye uptake and affinity of the dyes used were qualitatively similar to those reported for polynosic fiber at about 10% weight loss.[6] It is thought that the molecular aggregate in UE fiber at about 20% weight loss is similar to that in cellulase-treated polynosic fiber at about 10% weight loss.

At low weight loss, uptake of Blue 19, dye A, and dye B rapidly increased. We considered the possibility that dyeing with reactive dye may be difficult unless impurities, which are less active to reactive dyes, are removed. The primary wall and the matrix between fibrils in the secondary wall were known to contain impurities such as polysaccharides.[17] This will be discussed in detail later in reference to scanning electron microscopy (SEM) micrographs.

Dyeability of Mercerized Cotton Fiber Treated with Cellulase

The affinity of Congo Red to M fiber was 8.97 kcal/mol, which is higher than the affinity 8.33 kcal/mol to U fiber, when the affinity was calculated with a constant volume value of 0.22 L/kg. When the affinity of Congo Red to the cellulose molecule was 8.33 kcal/mol and the change in the affinity due to the cellulase treatment was assumed to be caused by the change in volume,[1,7] the volume of M fiber was calculated to be 0.32 L/kg. The value given in the literature[18] for volume of the mercerized cotton is 0.26 L/kg, which was measured by dyeing with one of the direct dyes.[18]

Uptake of two of the reactive dyes by M fiber was observed to be about two-fold higher than that by U fiber. It was clear that slack mercerization enhanced the number of regions dyeable by Congo Red and the reactive dyes used.

ME fiber showed different affinities compared to UE fiber, as shown in Figure 4. The affinity of Congo Red decreased at low weight loss, followed by a peak at 25.0% weight loss, and again became a minimum value at 32.7% weight loss. Finally, the affinity increased again to attain a value similar to that for U fiber. The volume ratio of the smallest value of 0.27 L/kg in ME fiber at weight loss of 32.7% to 0.32 L/kg in M fiber at weight loss of 0% was 0.84. This was larger than the 0.77 calculated for U and UE fibers. This finding was noteworthy because it was previously believed that mercerization enhanced the accessibility of the fiber to the enzyme. Respective minimum volume values of UE and ME fibers were 0.17 and 0.27 L/kg. Based on these values, we assumed

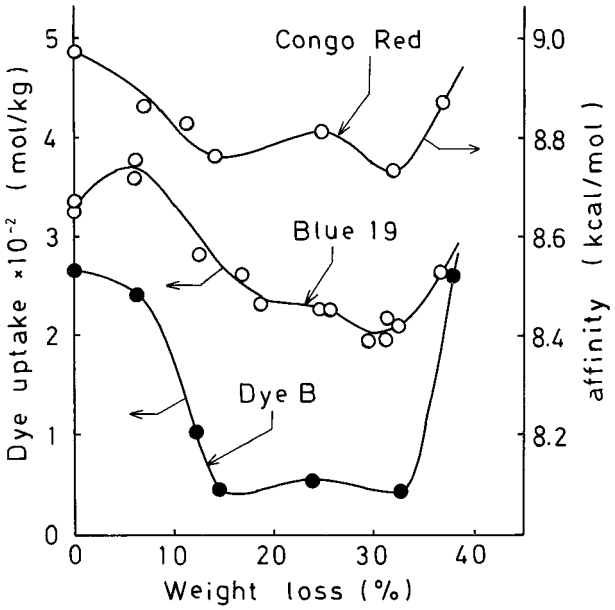

Figure 4 Uptake of two reactive dyes and apparent affinity of Congo Red versus weight loss caused by cellulase treatment of the mercerized fiber.

that the molecular aggregate in ME fiber is more easily dyeable by Congo Red than that in UE fiber even after cellulase treatment.

Uptake of dye B by ME fiber first gradually decreased and then rapidly decreased when weight loss exceeded about 10%. Uptake of dye B was low from about 15% weight loss up to about 32% weight loss and then increased abruptly and attained a value similar to that for M fiber. Uptake of Blue 19 by ME fiber first gradually increased and then decreased up to about 32% weight loss. After this, dye uptake increased again. The ratios of minimum dye uptake by ME fiber to that by M fiber were 0.6 and 0.2 for Blue 19 and dye B, respectively.

It was evident that the change in uptake of reactive dyes with cellulase treatment was more marked than the change of volume of Congo Red. The molecular size of dye species greatly influences the dyeing properties.[6] The molecular weight of dye B is about twice that of Blue 19 or Congo Red.[6]

Generally, the dependences of dye uptake or the affinity on weight loss were similar for all dyes used. In the previous article,[6] it was reported that cellulase-treated cupra fiber showed similar dependence of dye uptake or the affinity on weight loss, regardless of dye species. Accordingly, ME fiber showed changes in dye uptake and affinity similar to those for cupra fiber with increasing weight loss.

Change in the Crystallinity of Cellulase-Treated Fibers with Dyeability

X_c and I_c of U fibers with various weight losses are shown in Figure 5. In the case of U fiber, a peak appeared at about 10% weight loss for both X_c and I_c. These peaks indicate extensive hydrolysis of the disordered region at this weight loss. This interpretation is supported by the appearance of the minimum affinity of Congo Red at the same weight loss, as shown in Figure 3. After these peaks appeared, X_c decreased monotonically with weight loss, while I_c increased again up to 25.0% weight loss after reaching a minimum at weight loss of about 18%. The increase in I_c indicated that some ordered regions developed.[6] The increases in uptake (or affinity) of dye A and dye B (or Congo Red) around 20% weight loss were coincident with the decrease in X_c at the same weight loss.

X_c and I_c of M and ME fibers versus weight loss are also shown in Figure 5. Both X_c and I_c of M fiber were smaller than those of U fiber. The crystallinity of cotton fiber is known to be reduced by mercerization.[19] The two crystallinity indices exhibited here showed similar trends with increasing weight loss. There were minima at about 10% weight loss and peaks at about 25% weight loss for both X_c and I_c. These peaks were followed by decreasing trends for both X_c and I_c.

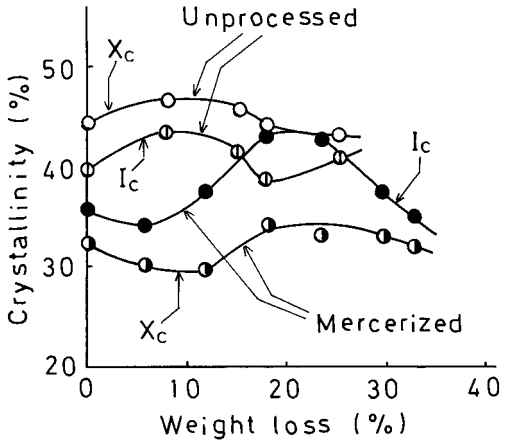

Figure 5 Crystallinity of the unprocessed and mercerized cotton fibers versus weight loss caused by cellulase treatment. Crystallinity indices X_c and I_c were obtained using XRD and infrared spectra, respectively.

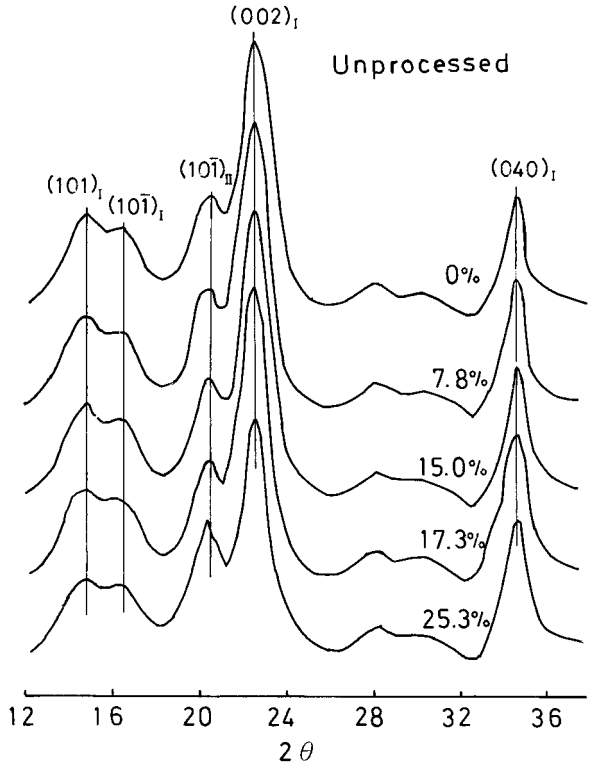

Figure 6 XRD patterns of the cellulase-treated unprocessed cotton fiber for the percentage of weight loss indicated. The sample with 0% weight loss is the starting material for cellulase treatment. Subscripts I and II of planes indicate cellulose I and cellulose II, respectively.

The decrease in crystallinity enhanced the dyeability of the fiber, and the increase in crystallinity reduced the dyeability of the fiber, as expected from results in previous articles.[1,6,7] Low dye uptake and affinity were found in the weight loss range of about 10 to 30%. This range was roughly consistent with that of higher X_c and I_c. Accordingly, the crystallinity was closely related to the dyeability of M and ME fibers.

XRD patterns of UE and ME fibers are shown in Figures 6 and 7, respectively. U and M fibers are also shown here for comparison. It was inferred that the cellulase treatment caused almost no change in the position of 2θ reflections from (101), (10$\bar{1}$), (002), and (040) planes of cellulose I.

The peak height of reflections from (10$\bar{1}$) and (002) planes of cellulose II changed markedly for ME fibers with weight losses of 5.8, 11.8, and 32.7%, as shown in Figure 7. For ME fiber with a weight loss of 32.7%, which showed minimum values of dye uptake or the affinity, the position of the 2θ reflection from the (002) plane of cellulose II clearly shifted to a position near that of the (002) plane of cellulose I. However, the reflection from the (10$\bar{1}$) plane of cellulose II was clearly observed for this ME fiber. Furthermore, no increase in the amount of cellulose I could be confirmed using DRIFT[20] and Raman spectra.[14]

Schurz et al.[21] treated bleached spruce sulfite pulp with *Trichoderma reesei,* assuming that cellulase could reach the surface of the fibrils but could not penetrate into the disordered regions within the fibrils and crystallites. One reason for this assumption was the observation of constant crystallinity with cellulase treatment time.[21]

This study shows that the cellulose I portion was more stable against cellulase treatment, even though crystalline disorder was introduced to this portion by mercerization. It is probable that the

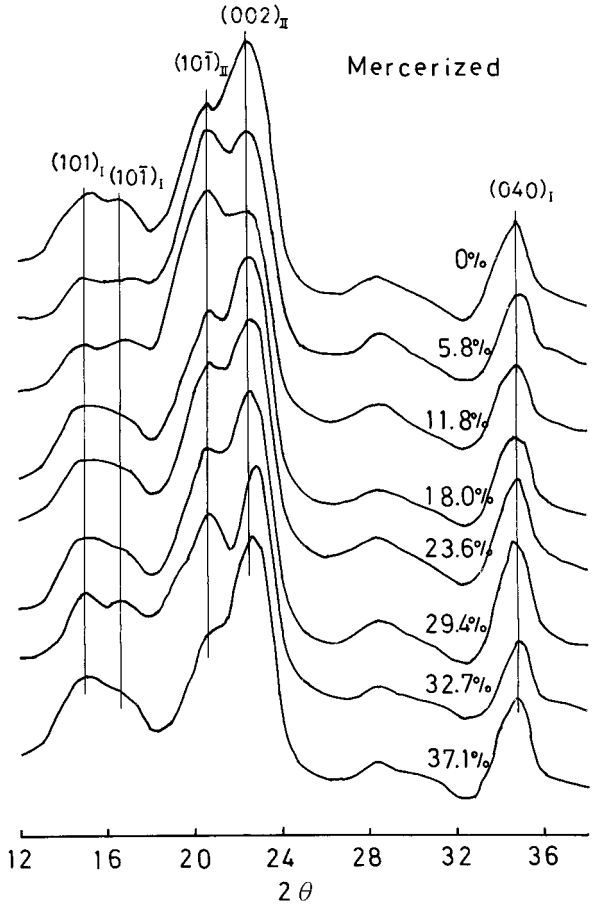

Figure 7 XRD patterns of the cellulase-treated mercerized cotton fiber for the percentage of weight loss indicated. The sample with 0% weight loss is the starting material for cellulase treatment. Subscripts I and II of planes indicate cellulose I and cellulose II, respectively.

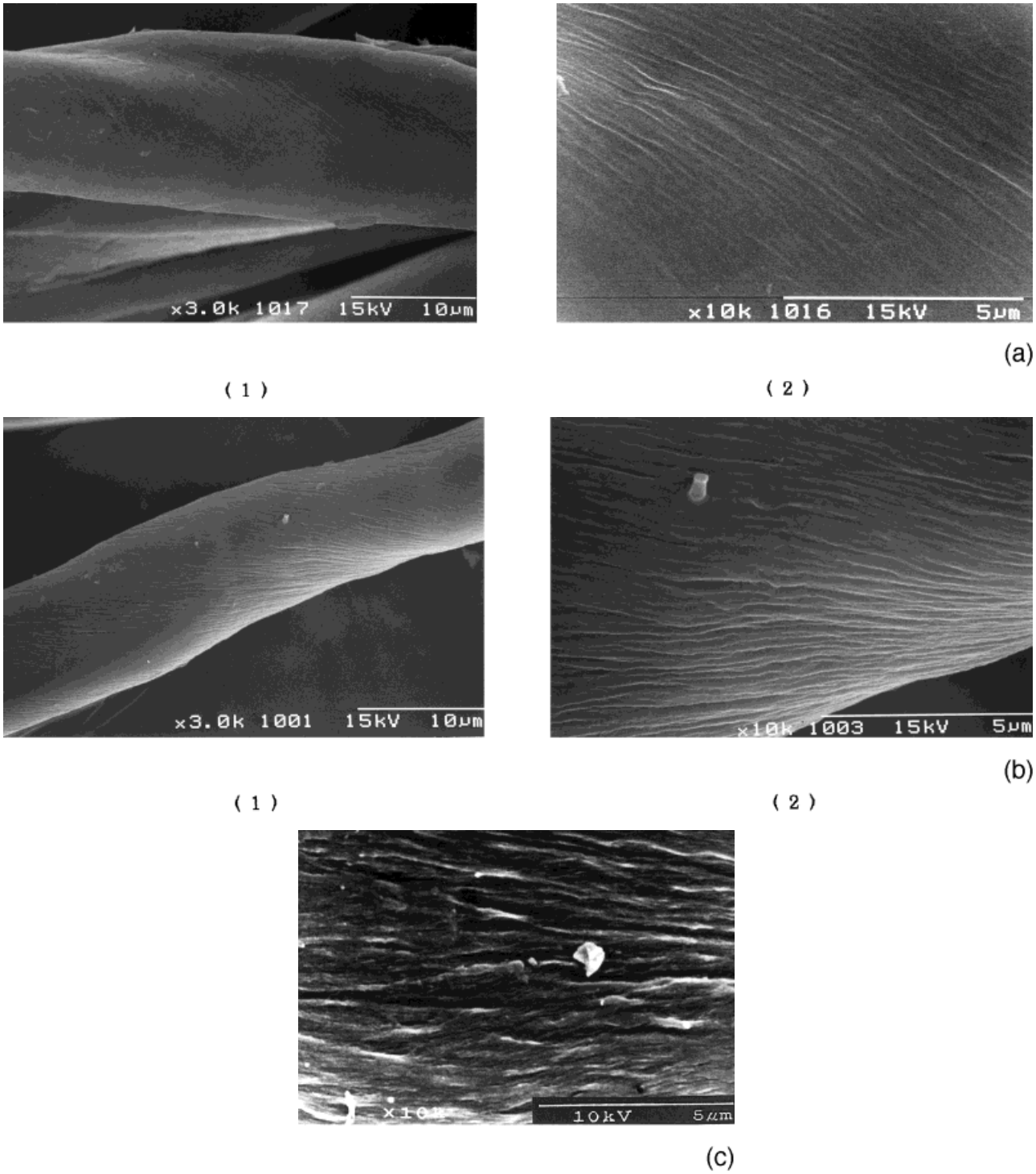

Figure 8 (a) SEM micrographs of the unprocessed cotton fiber: (2) is at higher magnification than (1). (b) SEM micrographs of the unprocessed cotton fiber with weight loss of 9.4% treated with cellulase: (2) is at higher magnification than (1). (c) SEM micrograph of the unprocessed fiber with weight loss of 17.3% treated with cellulase.

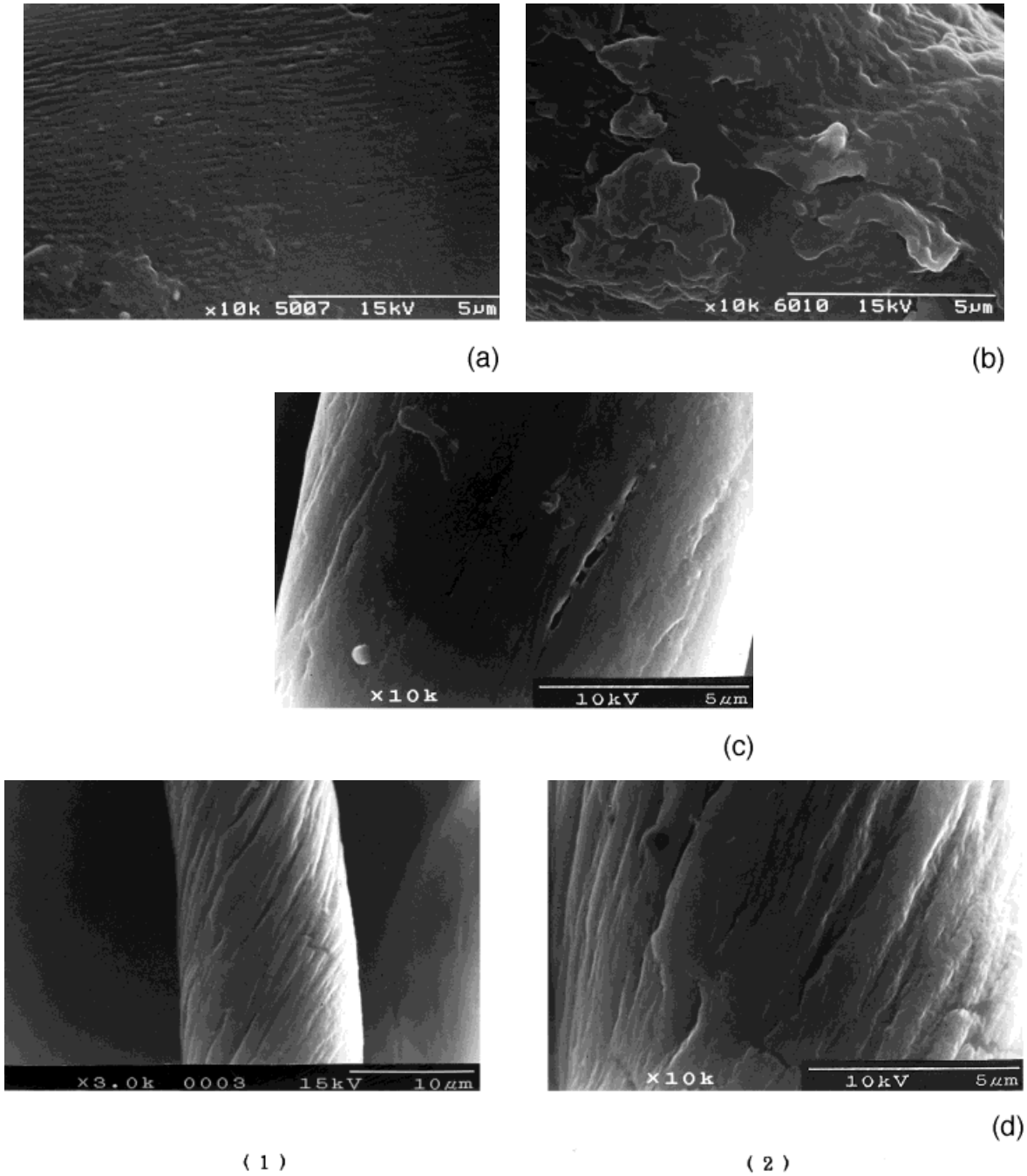

Figure 9 SEM micrographs of (a) the mercerized cotton fiber, (b) the mercerized cotton fiber with weight loss of 6.1% treated with cellulase, (c) the mercerized cotton fiber with weight loss of 25.0% treated with cellulase, and (d) the mercerized cotton fiber with weight loss of 37.1% treated with cellulase [(2) is at higher magnification than (1)].

disordered region within the fibrils and crystalline regions for M fiber was easily attacked by the enzyme. The influence of cellulase treatment on M fiber with crystalline regions must be studied in detail in the future.

Electron Microscopy

U fiber and UE fiber with 9.4 and 17.3% weight losses were observed by SEM, and the results are shown in Figure 8. It is difficult to see the fibrils

clearly on the surface of U fibers (1) in Figure 8(a). This is because the surface was covered with the primary wall, which contains a higher percentage of pectin and hemicellulose than the secondary wall. The primary component of pectin is galacturonic acid.[17] Galacturonic acid has a COOH group at C_6 of the hexose sugar. On the other hand, hemicellulose contains xyloglucan in which xylose residue is attached to C_6 position of glucan backborn. These components in pectin and hemicellulose are not highly reactive with the functional group of reactive dyes. The CH_2 OH at C_6 is known to be highly reactive with reactive dyes.[22]

It was clear that U fiber as a starting material had already undergone alkali treatment because the crystalline reflection of cellulose II was observed in Figure 2. Therefore, it is not surprising that the fibrils in the secondary wall are visible under high magnification, as shown in (2) in Figure 8(a). We confirmed, from (1) in Figure 8(a) that these fibrils were oblique to the fiber axis in U fiber.

Fibrils of UE fiber with 9.4% weight loss, shown in (1) in Figure 8(b) and (2) in Figure 8(b), are more distinct than those of U fiber. This means that cellulase treatment removed most of the primary wall, as well as the materials present between the fibrils in the secondary wall. This is especially apparent in (2) in Figure 8(a). It has been reported that there is a higher amount of pectin and hemicellulose in the matrix between the fibrils than within the fibrils.[17] Figure 8(b) supports the assumption that the increase in uptake of reactive dyes at low weight loss, as shown in Figure 3, is caused mainly by the digestion of impurities, such as polysaccharides, which cannot react with reactive dyes.

There are many cracks between the fibrils in UE fiber with 17.3% weight loss, as shown in Figure 8(c). Also, the orientation of the fibrils in this UE fiber was less ordered than that in U fiber. This suggests that enzymolysis proceeded within the fibrils, which is coincident with Paralikar and Bhatawdekar's observation that fibrils in cotton fiber were hydrolyzed into short fragments upon cellulase treatment.[23] The fibrils of cotton fiber were clearly swollen after the C_1 enzyme treatment. A similar observation was also reported by Takai et al.[24] Based on the observations in the present study, the enzyme action on U fiber was concluded to be not only interfibril but also intrafibril.

The surface of M fiber appeared smooth, and its texture was difficult to discern. However, some distinct fibrils in the fiber can be seen at high magnification, as shown in Figure 9(a). The fibrils are clearly swollen compared with the fibrils in U fiber shown in (2) in Figure 8(a). In spite of this swelling, the diameter of these fibrils is approximately 0.4×0.4 μm,[2] which is similar to that reported by Frey-Wyssling[25] for U fiber.

Micrographs of ME fiber with weight losses of 6.1, 25.0, and 37.1% are shown in Figures 9(b)–(d), respectively. ME fiber with 6.1% weight loss had a rough surface probably due to the destruction of the primary wall.

The surface of ME fiber with 25.0% weight loss was relatively smooth. This suggests that the primary wall was removed completely and that the fibrils in the secondary wall were more swollen than those of M fiber. Since the crystallinity of the primary wall is known to be lower than that of the secondary wall, the observation of the removal of the primary wall was coincident with the maximum of each X_c and I_c with about 25% weight loss. ME fiber with 37.1% weight loss had large cracks oblique to the fiber axis, as shown in (1) in Figure 9(d) but we could not discern individual fibrils at high magnification in (2) in Figure 9(d). This observation was closely associated with the fact that the fibrils including the crystalline region were swollen due to both the mercerization and the C_1 enzyme treatment.

CONCLUSION

The unprocessed cotton fiber was compared with a mercerized one in terms of weight loss caused by enzymolysis. Mercerization not only resulted in increased accessibility of fiber to cellulase and dye molecules but also introduced disorder in the crystallites. The highly ordered structure of cotton fiber was assumed to play an important role in the dyeability changes occurring with weight loss. The dyeability of the unprocessed cotton was dependent upon molecular aggregate, as was that of polynosic fiber, with a certain weight loss. The change in the dyeability of the mercerized fiber was closely associated with crystallinity and was similar to the case of cupra. The fibrils in the secondary wall were swollen due to the mercerization and also to the C_1 enzyme treatment. We observed large cracks but not individual fibrils for the mercerized cotton with high weight loss. The primary wall was digested in the initial step of hydrolysis in both the unprocessed and the mercerized fibers.

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