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Cellulase Dissolution Technique for the Study of Chemically Modified and Crosslinked Cotton

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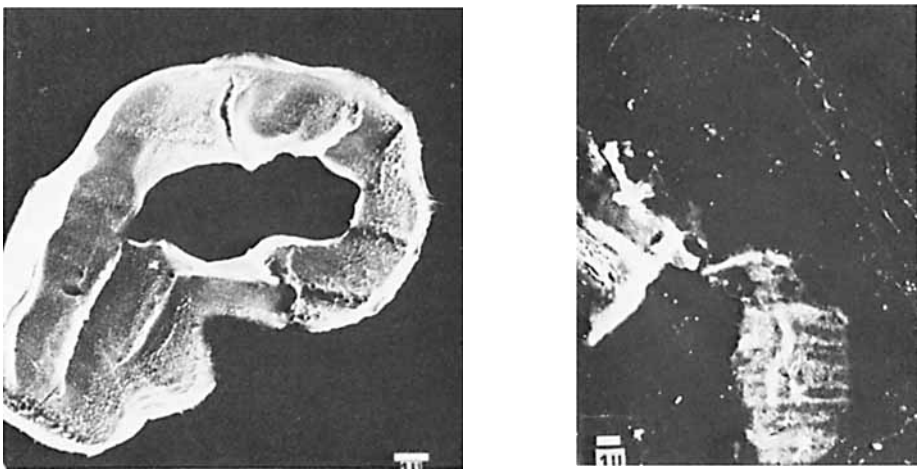
Synopsis

Cellulase enzyme of *Penicillium funiculosum* has been used for microsolubility test and compared with cuene dissolution technique by carrying out electron-microscopic observations on the acetylated and DMDHEU-crosslinked cottons. Cellulase being highly specific toward native cellulose, it acts only on unacetylated or uncrosslinked portions of the cotton leaving the rest of the cross section undisturbed. In this regard, cellulase dissolution technique has an advantage over the cuene dissolution technique currently used to evaluate modified and crosslinked cottons.

INTRODUCTION

Immersion in cupriethylenediamine hydroxide (cuene), called the cuene dissolution technique, has been extensively used by Rollins and co-workers at the Southern Regional Research Laboratory, New Orleans, for the evaluation of crosslinked and grafted cotton cellulose.[1,2] This is essentially a microsolubility test, the objective of which is to evaluate the uniformity of treatment, location of reacted regions, and texture of cellulose residue at the ultrastructural level. However, cuene of 0.5*M* concentration used in such tests is a powerful swelling agent and it is needless to state that there is every possibility of its distorting the basic morphology and texture of the original modified, crosslinked, or grafted cotton sample, especially at low levels of modification, crosslinking, or grafting. A more specific cellulose-dissolving agent which would act on chemically modified or treated cotton without causing any distortion of its morphology would, therefore, be more welcome. The cellulase enzyme is indeed such a specific agent, and a preliminary note on the cellulase dissolution technique for the study of chemically modified and crosslinked cotton is presented here.

There are many fungi that can grow on cotton, but very few elaborate cell-free enzyme that can solubilize it. The strain of *Penicillium funiculosum* (F_4) isolated in our laboratory is one such truly cellulolytic culture.[3] Betrabet and co-workers[4] have recently demonstrated the enzyme hydrolysis of cotton and other crystalline cellulosic substrates by *P. funiculosum* cellulase. This enzyme is rich in C_1 factor which is essential to make the crystalline cellulose more responsive to the subsequent action of C_x component of the cellulase enzyme complex. Betrabet[5] has also demonstrated recently the enzymic



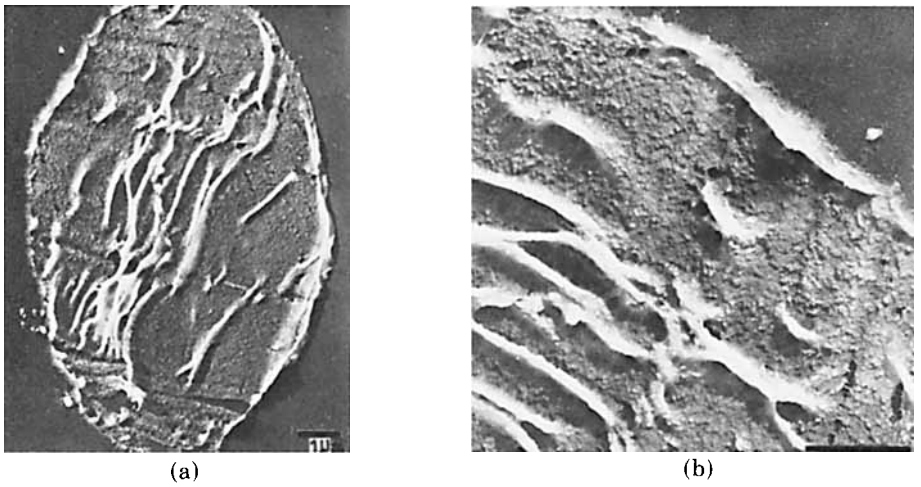
(a) (b)

Fig. 1. Electron micrographs of cross sections of cotton fibers: (a) untreated control; (b) section immersed in cellulase enzyme for 2 hr. Note the dissolution of cellulose and the effectiveness of the enzyme.

erosion of the ultrathin cross sections of normal cotton fiber viewed in the electron microscope.

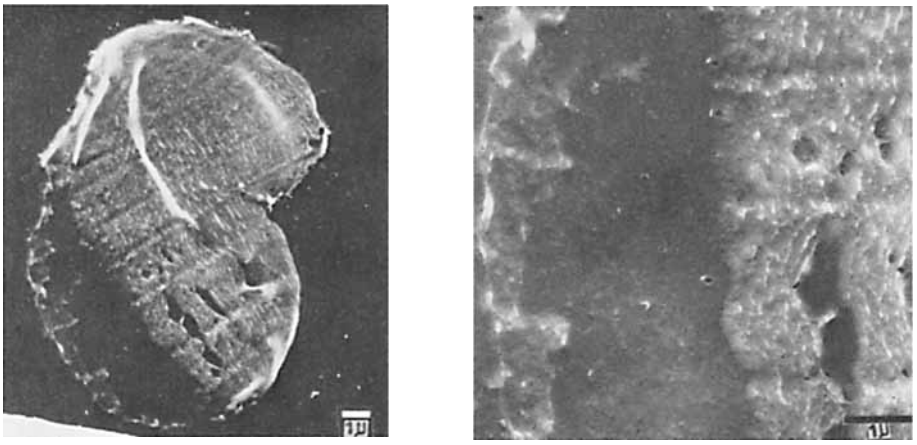
EXPERIMENTAL

The cellulase enzyme filtrate was obtained by growing *P. funiculosum* (F_4) in the manner described in an earlier publication,[4] and the enzymic activity was determined against cellulose powder.[6] The strength of the enzyme was adjusted to release 600 μg/ml of reducing sugar in terms of glucose in all the experiments conducted in the present study.



(a) (b)

Fig. 2. Electron micrographs of cross section of acetylated cotton of 0.88 D.S.: (a) low magnification; (b) high magnification. Note the crusty appearance of the section.

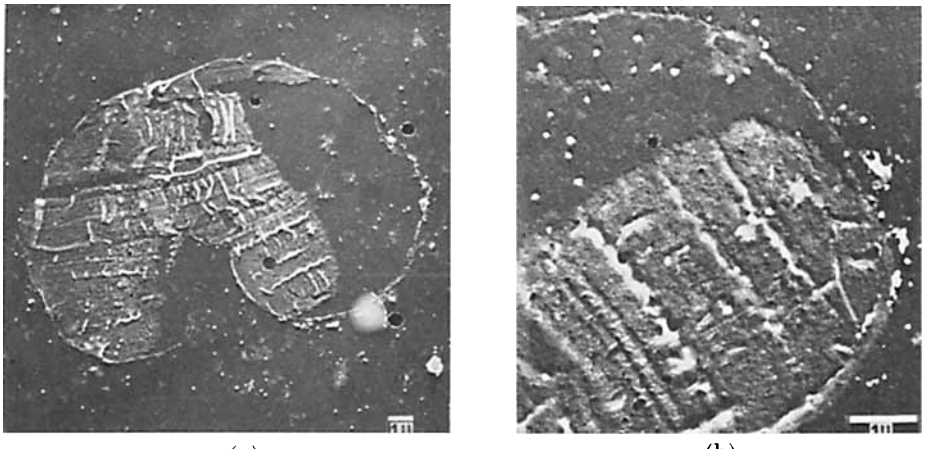


(a) (b)

Fig. 3. Electron micrographs of cross section of acetylated cotton of 0.88 D.S. immersed in cellulase enzyme for 1 hr: (a) low magnification; (b) high magnification. Note partial dissolution and softness of the texture as compared to Fig. 2.

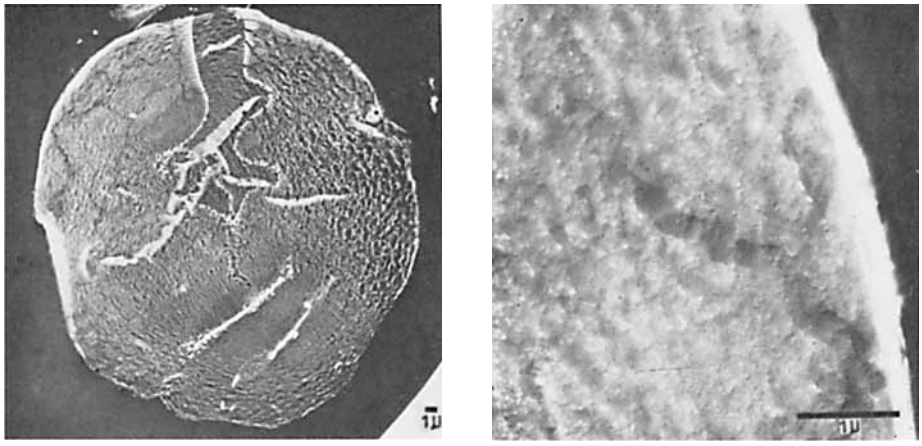
Purified cotton was acetylated by the procedure of Buras et al.[7] to obtain varying degrees of substitution, viz., 0.88, 1.77, and 2.40, by appropriately controlling the reaction time. The degree of substitution (D.S.) was calculated from the acetyl contents of the samples by the modified method of Eberstadt. [8]

Samples of crosslinked cotton varying in nitrogen content, viz., 0.42%, 0.71%, and 1.4%, were obtained by treating purified cotton with dimethyloldihydroxyethyleneurea (DMDHEU) adopting the method described by Gonzales and Benerito[9] using $MgCl_2$ as a catalyst and appropriately controlling the add-on. The Kjeldahl method was used to determine the nitrogen content.



(a) (b)

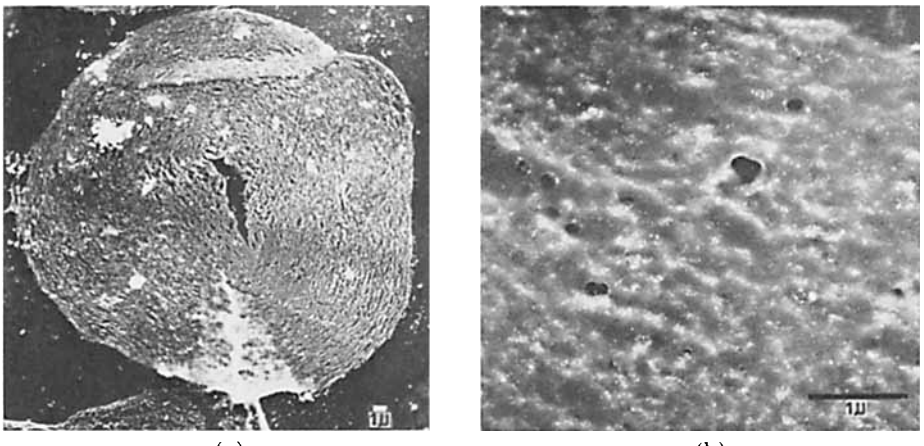
Fig. 4. Electron micrographs of cross section of acetylated cotton of 0.88 D.S. immersed in cellulase enzyme for 24 hr: (a) low magnification; (b) high magnification. Note close resemblance to Fig. 3 in spite of prolonged action of the enzyme.



(a) (b)

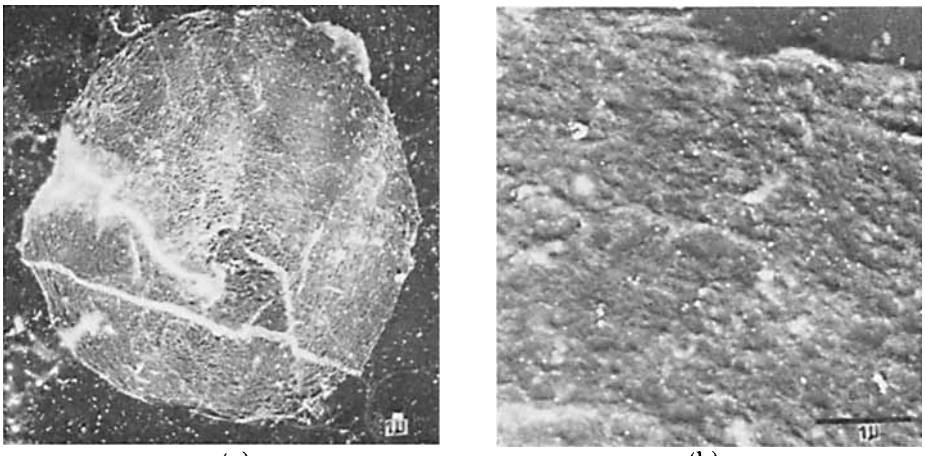
Fig. 5. Electron micrographs of cross section of acetylated cotton of 1.77 D.S.: (a) low magnification; (b) high magnification. Note the swollen and crusty appearance of the section.

The acetylated and crosslinked cottons were embedded in the prepolymerized mixture of methylbutyl methacrylates by the conventional flat embedding procedure.[10] A Porter-Blum ultramicrotome was used to cut about 500-Å-thin cross sections using glass knives. The sections were placed on the carbon-coated grids, and the embedding polymer was dissolved using methyl ethyl ketone. The grids were then immersed in a drop of enzyme solution adjusted to pH 5.6, using acetic acid–sodium acetate buffer and incubated at 50°C in an incubator. The incubation period was 1 hr, 2 hr, 4 hr, and 24 hr in the case of acetylated samples, and 1 hr, 6 hr, and 24 hr in the case of crosslinked samples. At the end of the incubation period, the grids were repeatedly dipped in distilled water to wash the sections free of the enzyme.



(a) (b)

Fig. 6. Electron micrographs of cross section of acetylated cotton of 1.77 D.S. immersed in cellulase enzyme for 1 hr: (a) low magnification; (b) high magnification. Note the close resemblance to the control, Fig. 5, and ineffectiveness of the enzyme.



(a) (b)

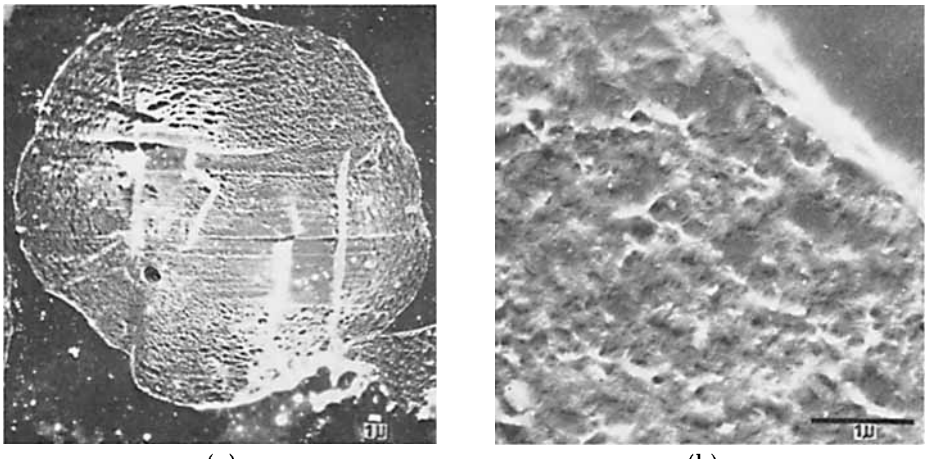
Fig. 7. Electron micrographs of cross section of acetylated cotton of 1.77 D.S. immersed in cellulase enzyme for 24 hr: (a) low magnification; (b) high magnification. Note the ineffectiveness of the enzyme in spite of prolonged action of the enzyme.

Simultaneously, a set of grids supporting the cross sections was treated with 0.5*M* cuene in the conventional manner[1,2] for comparison.

The cellulase- and cuene-treated cross sections were appropriately shadowed with platinum in a vacuum evaporator at an angle of about 25° and examined in a Hitachi electron microscope, HU 11E, at 75 KV both at low and high magnifications.

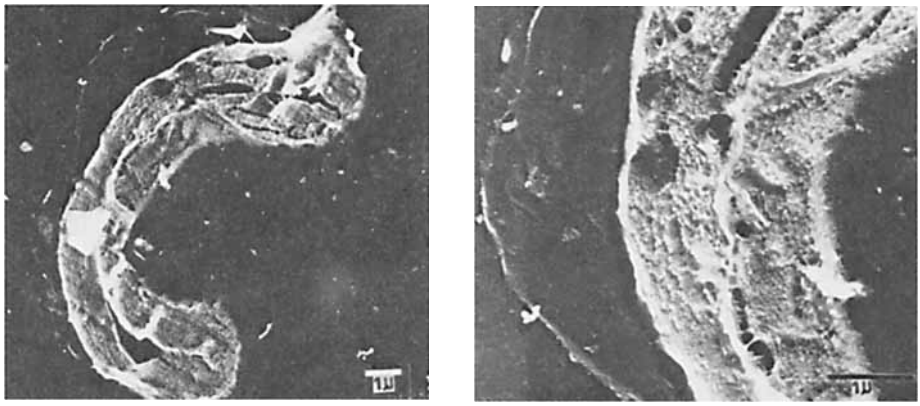
RESULTS AND DISCUSSION

The effectiveness of the cellulase enzyme used in the experiments was checked by treating the cross sections of normal cotton fiber (Fig. 1a) with



(a) (b)

Fig. 8. Electron micrographs of cross section of acetylated cotton of 1.77 D.S. immersed in 0.5*M* cuene for ½ hr: (a) low magnification; (b) high magnification. Note the indissolubility of the cross section and close resemblance to the enzyme treated cross sections in Figs. 6 and 7.



(a) (b)

Fig. 9. Electron micrographs of cross section of DMDHEU-treated cotton of 0.42% nitrogen immersed in cellulase enzyme for 1 hr: (a) low magnification; (b) high magnification. Note partial dissolution of the section.

the enzyme. It was confirmed that the enzyme dissolves most of the portion of the cross section (Fig. 1b), leaving some debris at the end of 2 hr.

A typical cross section of acetylated cotton of 0.88 D.S. is illustrated as a control in Fig 2. The enzyme action at the end of 1 hr causes considerable erosion, (Fig. 3a), and at a higher magnification (Fig. 3b), the softer texture can be seen more vividly in contrast to the crusty surface of the control (Fig. 2b). At the end of 2 hr, 4 hr, and 24 hr of exposure to the cellulase enzyme, there was only marginal increase in the dissolved portion as compared to the 1-hr enzyme-treated sample, indicating that 1 hr of enzyme treatment seems

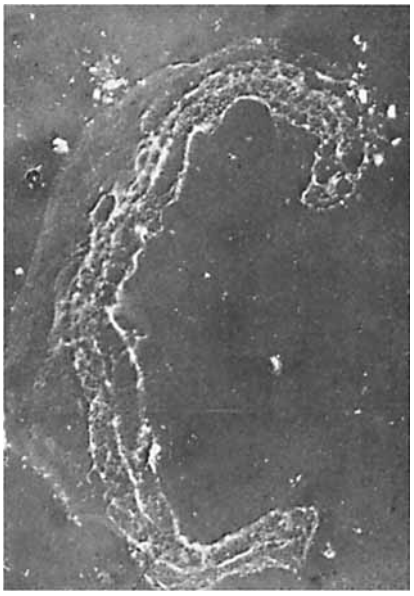
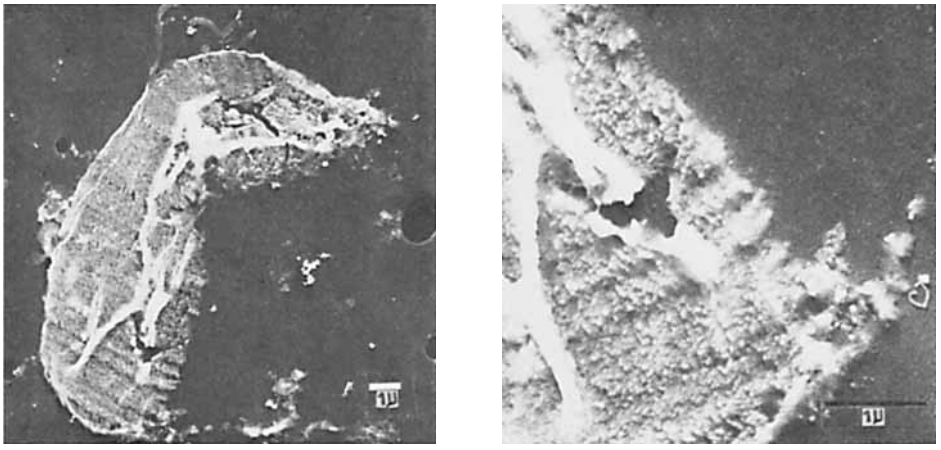


Fig. 10. Electron micrograph of cross section of DMDHEU-treated cotton of 0.42% nitrogen immersed in 0.5*M* cuene for ½ hr. Note the resemblance to cellulase-treated cross section in Fig. 9, but the action of cuene seems more drastic.



(a) (b)

Fig. 11. Electron micrographs of cross section of DMDHEU-treated cotton of 0.71% nitrogen immersed in cellulase enzyme for 1 hr: (a) low magnification; (b) high magnification. Note the partial dissolution of the section.

adequate to dissolve unacetylated cellulose. Fig 4, which is typical of a 24-hr enzyme-treated cross section, illustrates this point. These electron micrographs, as well as those that follow, also illustrate the highly resistant nature of the primary wall which remains as an almost intact border around the section. The electron micrograph of cuene-treated acetylated cotton cross section of 0.88 D.S. could not be taken as most of it had dissolved, leaving hardly any remnants.

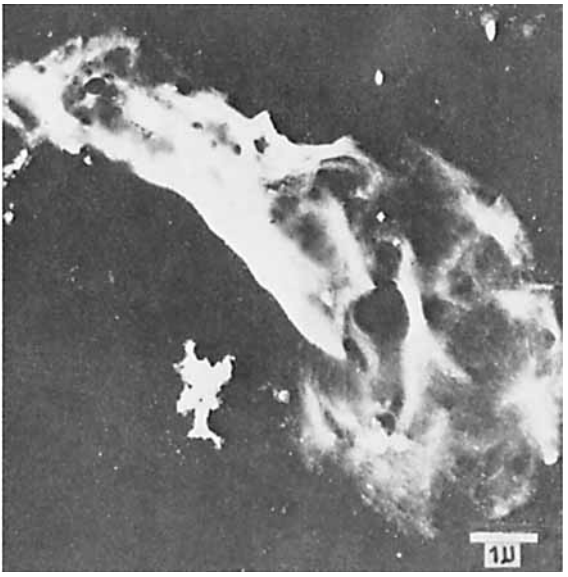
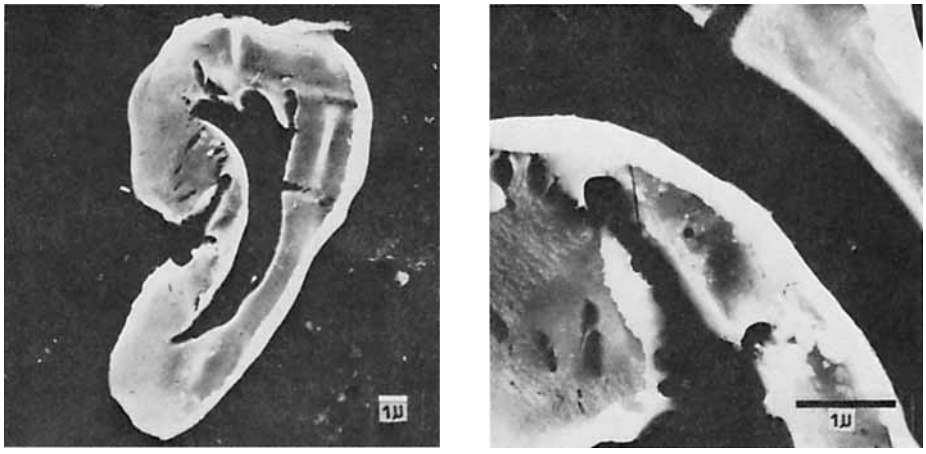


Fig. 12. Electron micrograph of cross section of DMDHEU-treated cotton of 0.71% nitrogen immersed in 0.5*M* cuene for ½ hr. Note partial dissolution of the cross section and distortion in the morphology due to swelling as compared to the cellulase-treated cross section in Fig. 11.

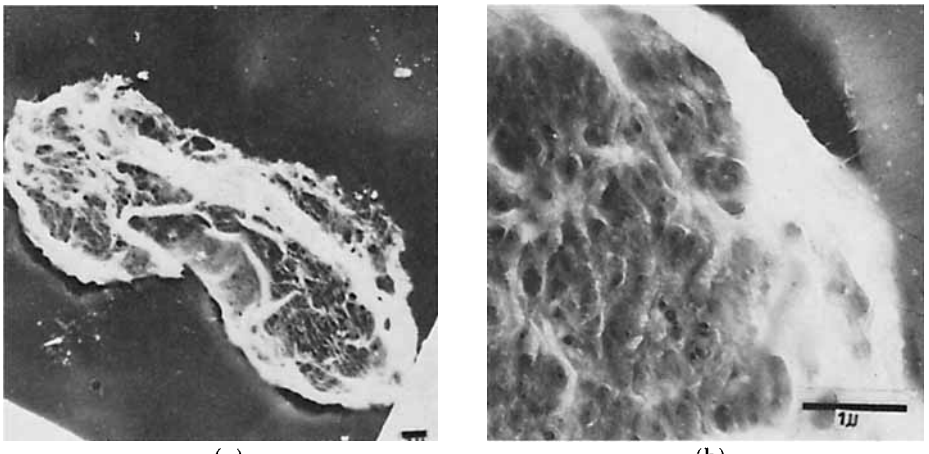


(a) (b)

Fig. 13. Electron micrographs of cross section of DMDHEU-treated cotton of 1.4% nitrogen immersed in cellulase enzyme for 1 hr: (a) low magnification; (b) high magnification. Note the ineffectiveness of the enzyme except for some peripheral cracks and erosion.

Figure 5 represents typical cross section of acetylated cotton of 1.77 D.S. The section reveals a layered structure due to the polymethacrylate used as an embedding material in the present study for convenience. Embedding in poly(vinyl alcohol) would have been ideal for acetylated cotton of higher D.S., but it is time consuming.[11]

The cellulase enzyme had hardly any effect on the layered morphology of acetylated cotton at the end of 1 hr (Fig. 6) or 24 hr (Fig. 7). Some softness in the texture and loss of crusty surface can, however, be observed at the higher magnifications, viz., Figures 6b and 7b. Cuene treatment, (Fig. 8)



(a) (b)

Fig. 14. Electron micrographs of cross section of DMDHEU-treated cotton of 1.4% nitrogen immersed in 0.5*M* cuene for ½ hr: (a) low magnification; (b) high magnification. Note the indissolubility of the cross section and resemblance to cellulase-treated cross section in Fig. 13, except that cuene seems to have disturbed the morphology.

confirmed the indissolubility of acetylated cotton of 1.77 D.S. At a still higher D.S. of 2.40, the cross sections of acetylated cotton were highly resistant to the action of cellulase enzyme or cuene as expected.

In the case of DMDHEU-treated cotton, the sample with 0.42% (Fig. 9) and 0.71% (Fig. 11) nitrogen content showed some susceptibility toward the cellulase enzyme; the sample with higher nitrogen content was somewhat more resistant. Cuene-treated cross sections confirmed these results (Figs. 10 and 12). The results obtained at the end of 1 hr of enzyme treatment alone are given since prolonged treatment for 24 hr made hardly any difference. Figures 9 and 11 show the dissolved portions and intact boundary of resistant primary wall, but the enzyme has not softened the crusty texture of the DMDHEU cotton as in the case of enzyme-treated acetylated cotton. Figures 10 and 12 illustrate that the action of cuene seems more drastic as compared with the morphology of cellulase-treated cross sections in Figures 9 and 11.

In the case of DMDHEU-treated cotton with higher nitrogen content of 1.4%, the action of the enzyme was mostly inhibited, except for some peripheral cracks and erosion (Fig. 13) with hardly any change in the original texture of the cross section. The cuene-treated cross section (Fig. 14) confirms this observation, but the inescapable conclusion is that the action of cuene seems rather violent which induces swelling and distorts the original morphology of the section.

CONCLUSIONS

The electron-microscopic examination of the cellulase-treated cross sections of acetylated and DMDHEU-treated cotton convincingly illustrates that the cellulase enzyme can indeed be used as a successful agent for microsolubility test. The enzyme, besides being highly specific toward unmodified cellulose, has added advantage inasmuch as its action is not violent and the morphology of enzyme-treated cotton at the ultrastructural level is more likely to be free from artifacts. This technique may also serve as a quick method to evaluate the cotton modified for imparting mildew or rot resistance.

The cellulase dissolution technique, however, needs further refinements and standardization with respect to the strength of the enzyme used and the exposure time of the treatment. The time of treatment could possibly be reduced to 30 min by increasing the strength of the enzyme. Its effectiveness also needs to be tested on a variety of chemically modified, crosslinked, and grafted cotton samples. Work in this direction is presently underway.

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