AFM SURFACE ANALYSIS OF FUNGAL MODIFIED CTMP FIBERS

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Fungal treatment can significantly improve the strength properties of chemithermomechanical pulp fiber, and it can help to expand the utilizations of the pulp. In this study, surface morphology of the eucalyptus chemithermomechanical pulp fiber before and after fungal treatment was studied with AFM. AFM phase images revealed that fungal treatment could remove part of lignin and extractives from fiber surface resulting in high carbohydrate content in the S_1 layer of the fiber. This observation was further supported by 3-D topograph AFM images.

Keywords: white-rot fungus, eucalyptus chemithermomechanical pulp, surface analysis, AFM

INTRODUCTION

Researches have indicated that biological treatment with enzymes, fungi and other bioactive substances can significantly increase the physical strength properties of CTMP, reduce the energy consumption in downstream refining and improve the drainability of pulp.[1-4] The treatment has attracted more and more attention due to its capability to reduce environmental pollution, energy consumption and fiber damage.

In our previous study,[5] it was found that the treatment of eucalyptus CTMP pulp with white-rot fungus *Trametes hirsuta* 19-6 for five days would improve the physical strengths of handsheets significantly. The tensile index, tear index and internal bond strength were increased by about 49%, 34% and 32%, respectively. SEM and TEM images showed that fungal treatment can degrade fiber wall material and significantly reduce the middle lamella remainder on the fiber surface. The removal of fiber wall material loosened the fiber wall structure or generated some weak points in the fiber wall. Therefore, the subsequent low-consistency refining amplified the fungal treatment effects, resulting in extensive internal fibrillation in the fiber wall, which improved the internal bonding between fibers. Although present research has shown the changes of fiber wall structure and fiber surface morphology after fungal treatment via SEM and TEM, the changes of CTMP fiber surface morphology in nanoscale are still not studied. In this paper, we further investigate the surface microstructure of CTMP fiber by atomic force microscopy (AFM) in order to unveil the details of fiber surface of the modifications of fiber surface by white - rot fungi treatment can be obtained.

EXPERIMENTAL

Materials

Eucalyptus CTMP pulps were obtained from Chinese Academy of Forestry, Nanjing Forestry Research Institute of Chemical Industry. Bio-CTMP pulp was prepared from eucalyptus CTMP pulps in the laboratory through the treatment with white-rot fungi 19-6 for 7 days.

AFM analysis

(1) Sample preparation. The eucalyptus CTMP pulps were diluted in deionized water and the fibers were fully disintegrated (the pulp consistency was controlled in less than 0.2%). The pulp suspension was then dropped on a double-sided adhesive tape affixed on the chip carrier, and the sample was naturally dried in a clean environment. During the drying process special caution should be taken to avoid sample contamination.

(2) AFM characterization. AFM characterization was performed using an MFP-3D™ atomic force microscope (Asylum Research, Inc.). The images were acquired in tapping mode in air using standard silicon AC160TS commercial digital instruments at a resonance frequency of about 250-300 kHz. The radius of the curvature of the used cantilever tips was set between 10-20 nm. Scanning frequency of 1.0-1.5 Hz and set point ratio between 600-800 mV were employed.

RESULTS AND DISCUSSION

Atomic force microscopy (AFM) is an effective technique for structural surface studies in the nanoscale. Applied in papermaking industry, it is mainly utilized to study the microstructures of fiber at the nanolevel. Usually AFM analysis provides two types of images, morphological images and phase images. Morphological images unveil the different surface morphology, structure and roughness, while phase images give surface information, such as different surface chemical composition, adhesion, flexibility and other properties.[6]

Chemical characterization of fiber surface

Although X-ray photoelectron spectroscopy (XPS) and secondary ion mass spectrometry (ToF-SIMS) are two most common techniques for surface chemistry study, AFM has been recently employed in the chemical characterization of fiber surface, besides its wide use in surface morphology study. It is well known that fiber surface mainly consists of three types of substances, namely, carbohydrates, lignin and extractives. Of these, lignin and extractives are hydrophobic, whereas carbohydrates are hydrophilic. Gustafsson *et al.*[6] suggested that hydrophobic and hydrophilic substances show different phases in AFM phase image. The different phases have different shades of color (or shading). Usually, the hydrophilic substances are darker due to their less adhesive interaction with the AFM probe. Comparatively, hydrophobic substances are generally lighter in color owing to their greater adhesive interaction with the AFM probe. Therefore, the chemical composition of fiber surface can be distinguished by comparing the AFM phase diagrams and the mechanical curve between the fibers and probe. Some studies found that lignin is usually in the form of patches or spheres in the AFM phase image;[7,8] other studies also found that lignin, extractives and hemicelluloses tend to form granules on fiber surface.[9]

Figure 1(a) and (b) exhibit the AFM phase images of unbleached eucalyptus CTMP pulps scanned on an area of 3.0×3.0 μm. As shown in Fig. 1(a), the fibrils do not have a fixed orientation, indicating that the scanned area belongs to the surface of a fiber separated from middle lamella. On the other hand, the fibrils displayed in Figure 1(b) are oriented, suggesting that the surface of the fiber observed is the S1 layer. In both images, the surfaces are covered with different size granular substances that display regular globular particles (rectangle tag) and irregular patches (oval tag). In general, the irregular patches are large in size. Although it is unclear what the exact chemical composition of those granular substances is, it is possible that they consist in lignin or extractives, as these are the two main components of middle lamella. Meanwhile, the particles covered on the S1 layer surface are probably the re-deposited lignin or extractives.[10] It may be thus concluded that CTMP pulp fiber, separated either from middle lamella or from secondary layer, is covered with a layer of granular substances that are probably lignin or extractives.

To investigate the chemical composition of those granular substances, acetone was used to extract the CTMP pulp fibers. Fig. 2 shows the AFM phase image of a CTMP fiber after extraction. As may be noted in the image, most of the regular globular particles are removed by the extraction, resulting in a much smoother surface. However, the extraction did not eliminate the irregular patches. It has been reported in some studies[10] that acetone extraction mainly removes the extractives, but does not significantly remove lignin from the CTMP fiber surface. It thus suggests that the regular globular particles covering the CTMP fiber surface are extractives.

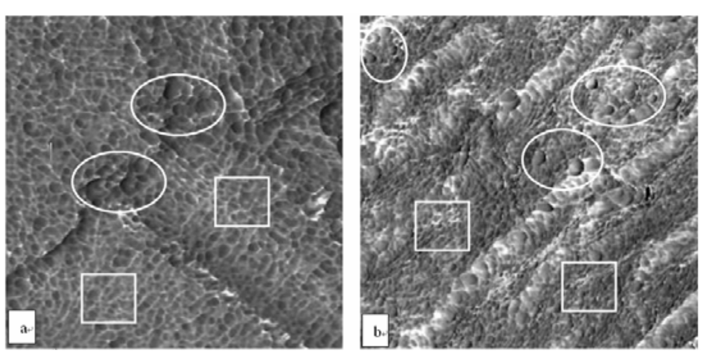

Figure 1: AFM phase image of unbleached Eucalyptus CTMP fiber separated from lamella (a) and from S1 layer (b)

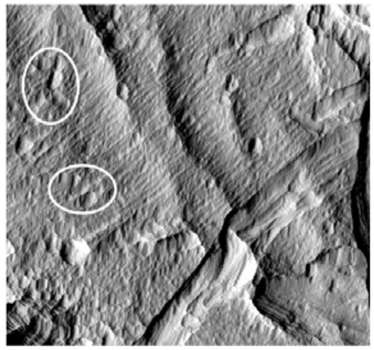

Figure 2: AFM phase image of unbleached eucalyptus CTMP fiber after acetone extraction

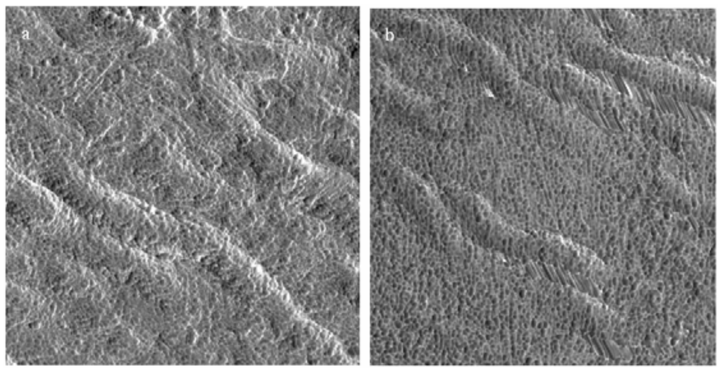

Figure 3: AFM phase image of eucalyptus CTMP pulp bleached by 1.5% H2O2 (a) and by 3% H2O2 (b)

Since it has been confirmed that the regular globular particles represent the extractive component, the irregular patches are possibly lignin. To investigate this, the CTMP pulp was bleached with H_2O_2. Fig. 3 displays two phase images of CTMP fiber after H_2O_2 bleaching. As shown in the two images, most of the irregular patches on the surface of the bleached CTMP fibers almost disappeared and only the small and regular globular particles remained on the fiber surface, suggesting that H_2O_2 bleaching could not significantly remove extractives from the CTMP fiber surface (which is in line with the conclusion of L. Böräs *et al.*[10]). Koljonen *et al*. confirmed that H_2O_2 bleaching could not remove lignin from the CTMP fiber surface and that lignin occurred in a non-granular form on the fiber surface.[11] Thereby, we propose that the irregular granular patches should be lignin. As shown in Fig. 3(a) and (b), the removing rate of irregular particles increased with the increase in the amount of H_2O_2. When the H_2O_2 dosage was 1.5%, there were still some irregular particles on the fiber surface (Fig. 3a), but the irregular particles basically disappeared (Fig. 3b) as the H_2O_2 dosage went up to 3.0%.

By this analysis, we managed to distinguish the chemical composition of the granular substances covering the CTMP fiber surface. The above analysis methods and theoretical basis are the foundation of using AFM to study the lignin and extractives of eucalyptus CTMP fiber surface before and after white-rot fungus treatments.

AFM analysis of white-rot fungus modified CTMP fiber surface morphology

Our previous studies have shown that the treatment with white-rot fungus can loosen the structure of CTMP fibers and peel off the primary layer, resulting in the exposure of the S1 layer, which is rich in cellulose.[5] In order to investigate this phenomenon further at a nanoscale, the changes in surface morphology of CTMP fibers before and after white-rot fungus treatment were studied by AFM.

Figures 4 and 5 present the AFM phase images of eucalyptus CTMP fibers before and after the white-rot fungus treatments. As indicated, for CTMP fibers separated from the middle lamella and the second layer, both regular and irregular granular substances were significantly reduced after 14 days of white-rot fungus treatment. Interestingly, the images display that not only extractives of regular globular form are removed, but also the lignin of irregular form is substantially eliminated. This suggests that white-rot fungi are able to degrade both lignin and extractives on the fiber surface. This observation is generally in agreement with the results of SEM and TEM analyses. Figure 5 reveals that the fiber separated from the secondary layer had remarkably less lignin and extractives on the surface as compared to the fiber separated from the middle lamella, which is because the lignin and extractives were not originally covered in the S1 layer, and the formation of the cover layer was mainly due to the fact that lignin and extractives dissolved and then deposited in the S1 layer during the CTMP pulping process.[10] After 14 days of treatment, most of the lignin and extractives on the S1 layer were removed, resulting in carbohydrate-rich fiber surface. This change in the chemical composition of the surfaces is, however, the primary reason for the improvement of the fiber bonding capacity induced by the white-rot fungus treatment.

As discussed by Gustafsson *et al.*,[6] the hydrophobic substances are darker in the AFM phase image due to their smaller adhesion force to the AFM probe. By contrast, hydrophobic substances are generally lighter in color due to their greater adhesion to the AFM probe. Therefore, we can estimate the relative proportions of hydrophobic and hydrophilic substances through the comparison of the phase difference (characterized by the color difference). In general, the more hydrophobic material covering the CTMP fiber surface, the greater the phase difference will be. Figure 6 shows the AFM phase diagram of CTMP fiber after the white-rot fungus modification. Diagram data show that the phase difference in the diagram of CTMP fiber before white-rot fungus modification is of 62 degrees, whereas that of CTMP fiber after white-rot fungus modification is of only 16 degrees, i.e. decreasing by 74%. It thus indicates that the white-rot fungus treatment can effectively remove lignin and extractives from the CTMP fiber surface.

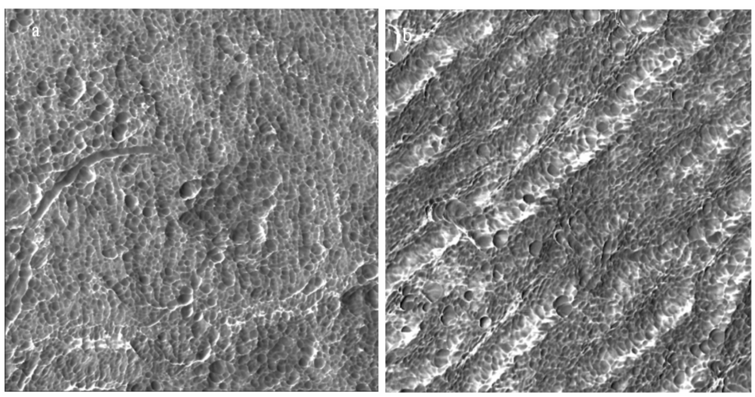

Figure 4: AFM phase diagrams of eucalyptus CTMP pulp before fungi treatment: (a) fiber separated from lamella and (b) fiber separated from layer S1 (scan area 3.0×3.0 μm)

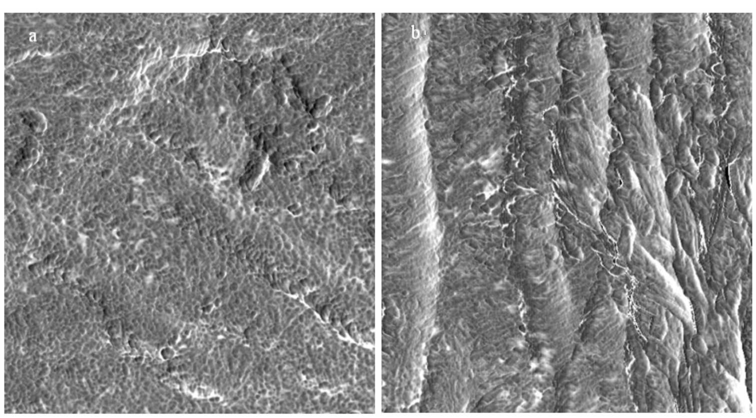

Figure 5: AFM phase diagram of eucalyptus CTMP pulp after 14 days of fungi treatment

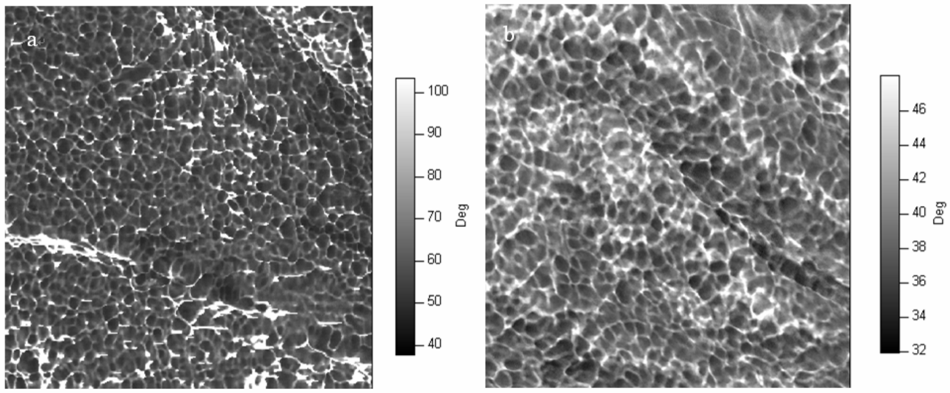

Figure 6: AFM phase diagram of CTMP fiber surface before and after white-rot fungus modification (scan area 1.0×1.0 μm)

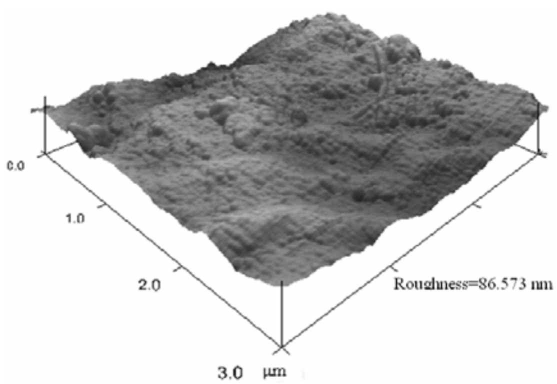

Figure 7: Surface topography of CTMP fiber without fungal modification

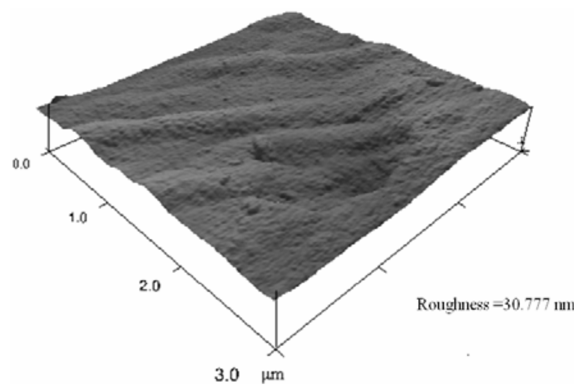

Figure 8: Surface topography of a CTMP fiber modified by fungi for 14 days

Topograph of CTMP fiber surface

AFM 3D topograph can reveal the topographical features of "peaks" and "valleys" on the scanned area. The results are often used to characterize the surface roughness of material. We applied this technique in the characterization of pulp fiber surface. Fig. 7 demonstrates the surface topograph of a CTMP fiber before white-rot fungus treatment, and Fig. 8 presents the surface topograph of a treated CTMP fiber. The scan areas of these two samples are of 3.0×3.0 μm.

The analysis of Figs. 7 and 8 indicates that the roughness of the CTMP fiber surface before the white-rot fungus treatment is of 86.573 nm, with a statistical area of 3.0×3.0 μm, whereas that of the treated CTMP fiber is of 30.777 nm. White-rot fungus treatment reduced the CTMP fiber surface roughness by 64.4%. This reduction in roughness is attributed to the removal of the granular substances on the fiber surface. Fig. 7 also displays a large number of "valleys" and "peaks", which indicate the presence of granular substances (i.e. lignin and extractives). After white-rot fungus treatment, the fiber surface became smooth, as shown in Figure 8. The AFM 3D topograph analysis thus further supports the contention that white-rot fungus treatment can eliminate parts of lignin and extractives, leading to a smoother fiber surface.

CONCLUSION

(1) The CTMP fiber surface is covered by a layer of granular substances present in regular and irregular form.

(2) The extraction, H_2O_2 bleaching experiments and AFM analysis have confirmed that the irregular patches on the CTMP fiber surface are lignin and the regular globular particles are extractives.

(3) By the analysis of AFM phase images, we found that white-rot fungus treatment can degrade and remove lignin and extractives from the CTMP fiber surface, resulting in a fiber surface that is rich in carbohydrates.

(4) 3D AFM topograph analysis illustrates that the degradation of lignin and extractives on the

CTMP fiber surface by white-rot fungus treatment leads to a decrease in the roughness of the fiber surface.

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