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Atomic Force Microscopy Characterization of Switchgrass

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Switchgrass, *Panicum virgatum,* is a native North American perennial grass with the ability to generate moderate to high yields on marginal farmlands. It is one of the target crops that are under development for production of ethanol from lignocellulosic biomass [1]. Lignocellulosic biomass is largely composed of cellulose, hemicellulose and lignin, which are closely associated with each other in the plant cell wall [2]. Deconstruction of the plant cell wall to enable conversion of the cellulose and hemicellulose to fermentable sugars is complicated by this laminate structure and the recalcitrance of its components, particularly lignin and cellulose. Cellulose is composed of crystalline microfibrils which are resistant to both chemical and enzymatic depolymerization. The chemical composition and structure of lignin strongly influence the recalcitrance of biomass to deconstruction and fermentation. In addition to its structural role in the cell wall, lignin impedes access of hydrolytic enzymes to cellulose and hemicellulose. Currently, energy-intensive thermal and chemical pretreatment methods must be employed to render lignocellulosic biomass easier for the enzymes to depolymerize for subsequent conversion to fuels [3]. Thus, a fundamental understanding of the structural changes and associations that occur at the nanoscale level during biosynthesis, deconstruction, and hydrolysis of biomass is essential for improving processing and conversion methods for lignocellulose-based fuels production.

In this study, we have used atomic force microscopy (AFM) to compare the structures of switchgrass samples that were subjected to various treatments that have been shown to remove specific components from lignocellulosic biomass[4].. Our samples include intact switchgrass, extractive-free switchgrass, holocellulose, and cellulose. The image of intact switch grass (not shown) has a smooth, waxy surface. The extractive-free switchgrass has been extracted with benzene and ethanol to remove lipid, protein, and oils. This sample is composed of cellulose, hemicellulose and lignin, and the image is shown in FIG. 1 (A). The holocellulose is the sample of extractive-free switchgrass in which lignin has been removed by treatment with glacial acetic acid and $NaClO_2$. The image of holocellulose, the switchgrass sample with only cellulose and hemicellulose, is shown in FIG. 1(B). The holocellulose sample was then treated with diluted acid, 2.5 M HCl, heated at 100C for 4 hours to remove hemicellulose from the cellulose. The image of cellulose is shown in FIG. 2. The image of switchgrass cellulose showed microfibrils similar to our previous images of cotton cellulose [5]. Compared FIG 2 with FIG 1(A), the cellulose, hemicellulose, and lignin are interlaced with each other smoothly. However, the removal of lignin in FIG 1(B), produced a relatively rough surface, and it is very different from pure cellulose in FIG 2. As shown in FIG. 1 (B), the hemicellulose is tightly associated around cellulose that the cellulose cannot be differentiated from hemicellulose by observation. Our images showed very different surface structures result from the various component-extractive treatments of switchgrass. AFM should be a useful tool for diagnostic examination of pretreatment procedures at various stages of conversion to fuels.

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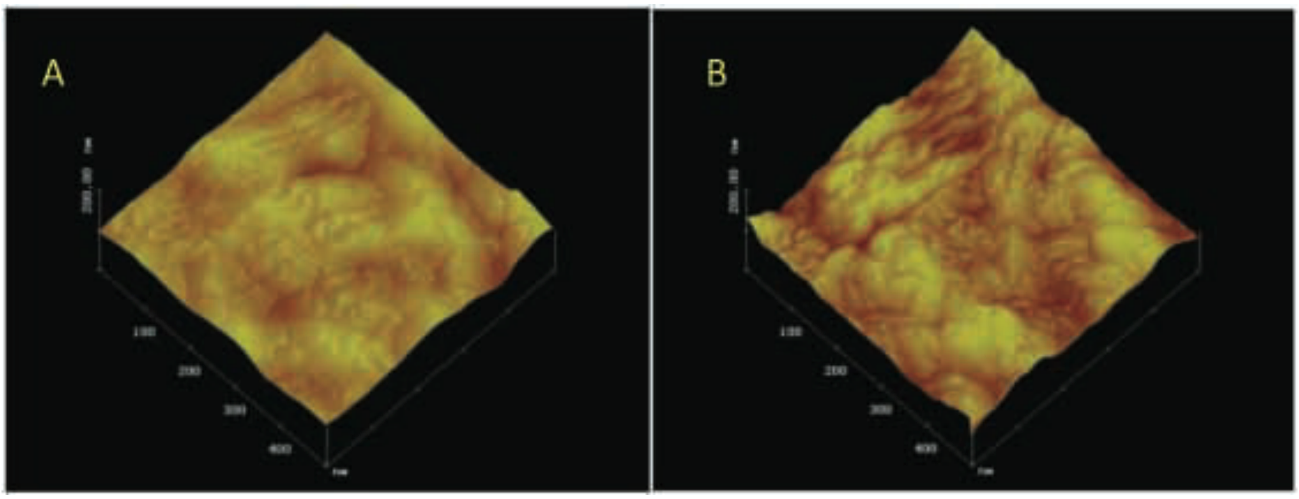

FIG. 1. (A) is AFM image of extractive-free switchgrass. (B) is AFM image of holocellulose.

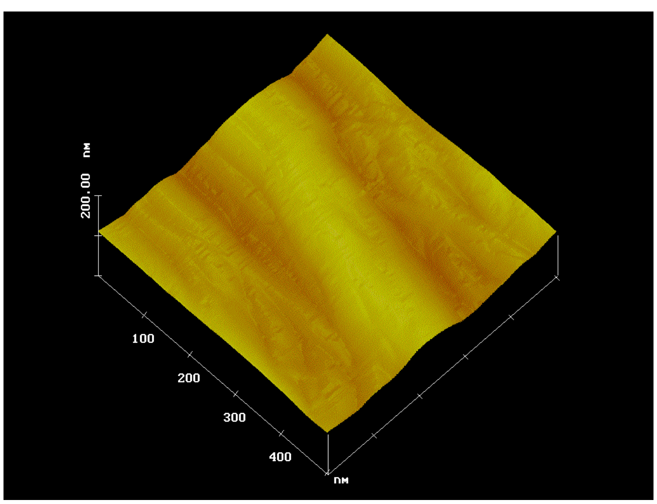

FIG. 2. AFM image of cellulose.

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