Surface Characterization of Lignocellulosic Biomass for Understanding the Enzymatic Hydrolysis

Jing Li, Baran Arslan, Xiao Zhang, Nehal Abu-Lail

NARA program, The Gene & Linda Voiland School of Chemical Engineering and Bioengineering, Washington State University, Pullman WA 99164

Abstract

To better understand the enzymatic hydrolysis of lignocellulose in the fundamental level, our research aims at characterizing the surface of biomass by visualizing the chemical properties of the model cellulosic substrates via force-volume mode of Atomic Force Microscopy (AFM) imaging. Characterizing the substrate surface at the nanoscale enabled us to determine the attractive interactions between the modified AFM tip and fibril surface due to the hydrophobicity of the reference substrates and methyl group.

Introduction

Lignocellulose, which consists of cellulose, lignin and hemicellulose, is regarded as one of the most promising potential sources for non-sugar supply and ethanol production. The production of biofuels from biomass passes by many steps, of which enzymatic hydrolysis of biomass is the hindering step. This is mainly due to the natural resistance which plant cell walls have to enzymatic deconstruction often referred to as biomass recalcitrance.

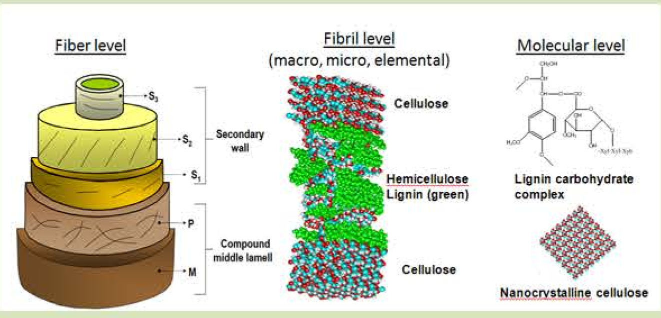


Figure 1. Different fiber structural levels

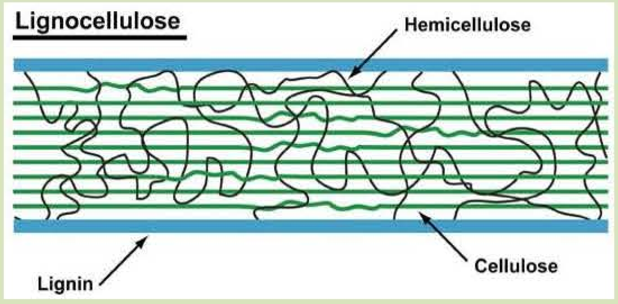


Figure 2. Lignin coverage of cellulose and hemicellulose structure.

- The cost of effective lignocellulose biomass conversion process is still prohibitive.
- Fraction of three main components: 35%-50% cellulose, 20%-35% hemicelluloses (xylan) and 15%-20% lignin (1)
- Cellulose contains abundant glucose chains that can be broken apart by enzymatic hydrolysis.
- Improving the enzymatic hydrolysis efficiency is dependent on understanding of the biomass characteristics such as lignin contents, available surface area of substrates, degree of polymerization and cellulose crystallinity (2).

Method & Experiment

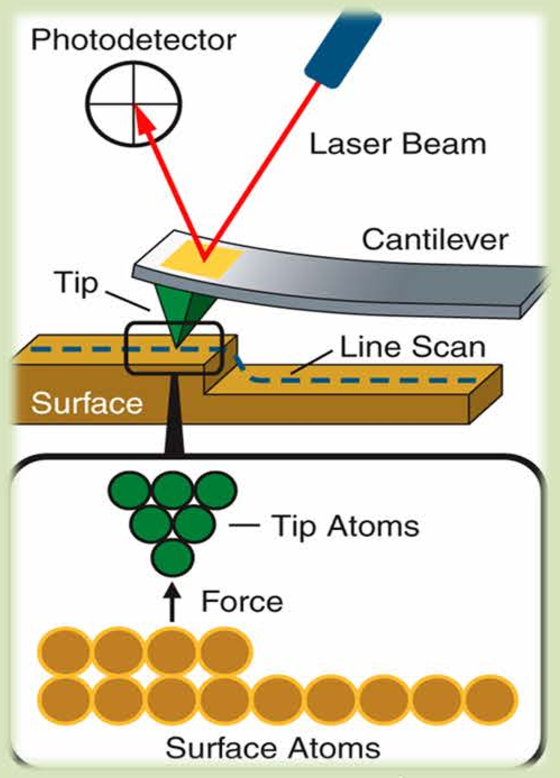


Figure 5. AFM rationale

Figure 6. AFM components

- Gold-coated cantilever tip is modified in the solutions with three functional groups

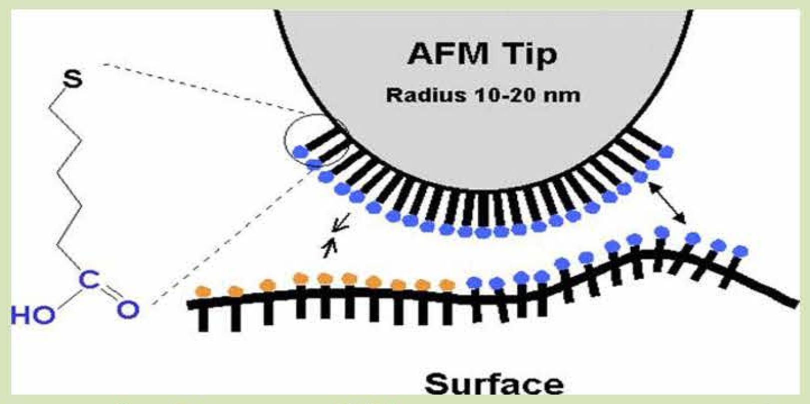


Figure 7. Chemical force microscopy rationale

- Atomic Force Microscopy contact mode
- Capture Fore-Volume(FV) images once deflection/force curves are stabilized

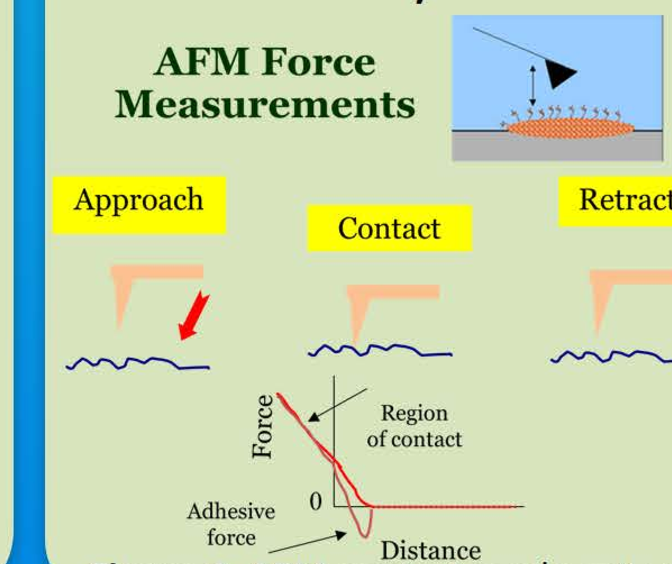


Figure 8. AFM contact mode rationale

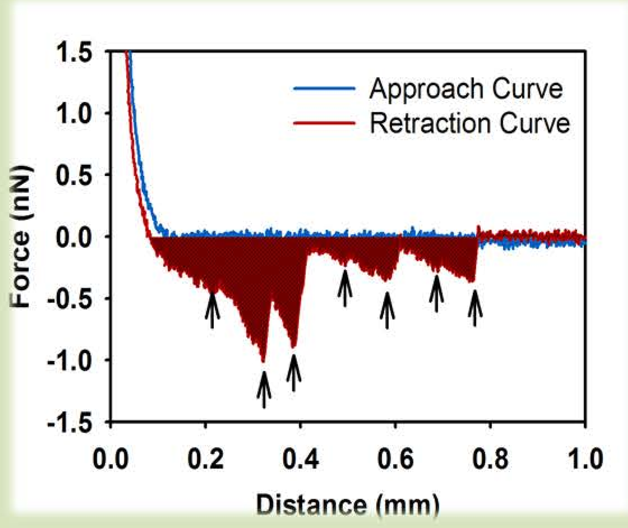
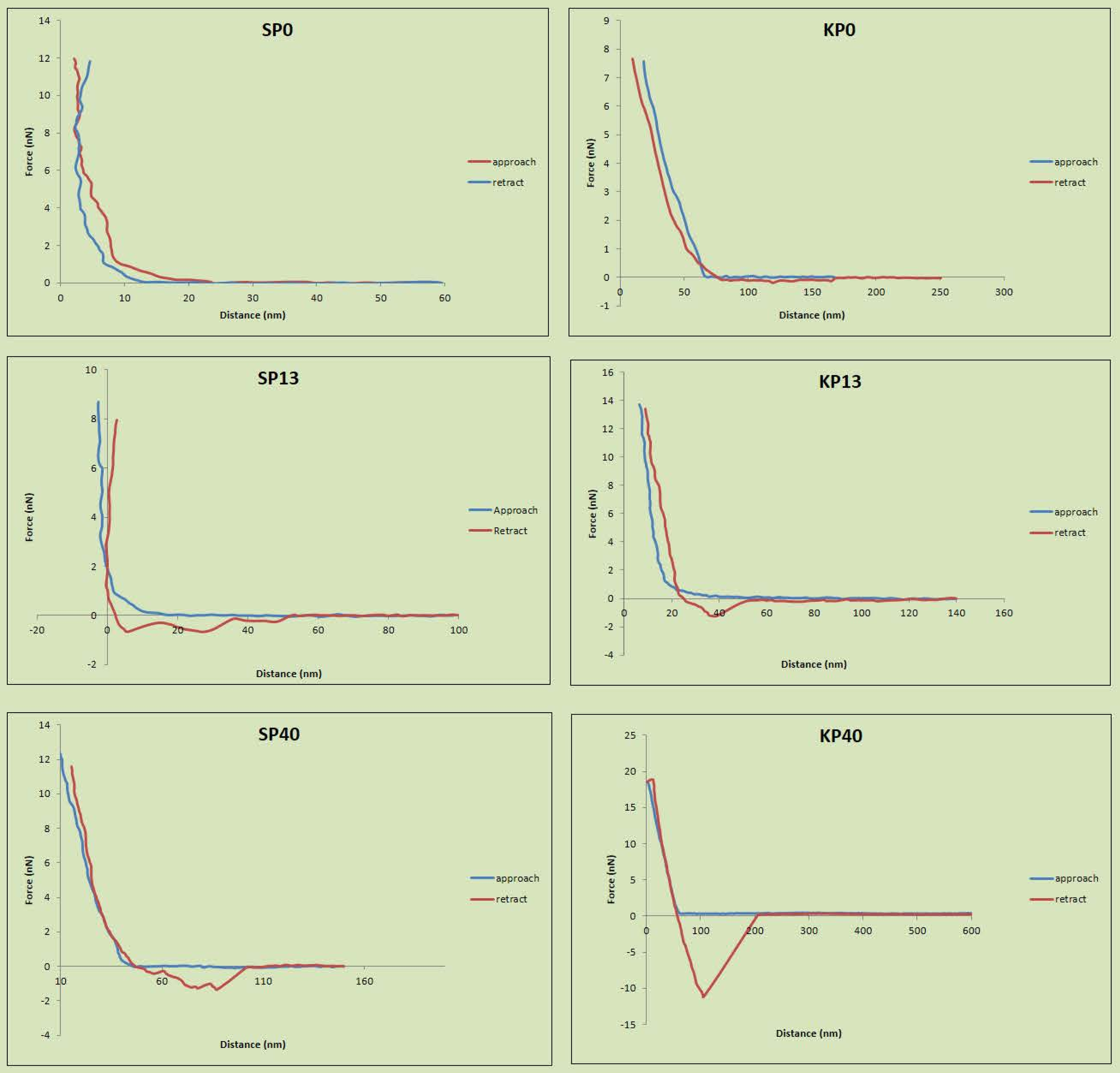


Figure 9. Approach/retraction curve

Results



Data Analysis

- SP0 < KP0 (lignin free) barely have any interaction with methyl group
- SP13 < KP13 display some attractive forces with methyl group
- KP13: approximately 1 nN from sample results
- SP40 < KP40 have the strongest interaction forces
- KP40: approximately 12 nN from sample results

Materials

- Reference substrates with controlled fiber properties and chemical composition are provided by chemical pulping methods.
- All substrates derived from poplar.
- KP=kraft pulping SP=sulfite pulping

$HSCH_2(CH_2)_8CH_2COOH$ 11-Mercaptoundecanoic acid 95%

$HSCH_2(CH_2)_9CH_2OH$ 11-Mercapto-1-undecanol 97%

$CH_3(CH_2)_{16}CH_2SH$ 1-Octadecanethiol purum, ≥95.0% (GC)

Table 1. Chemical composition and surface characterization of reference substrates

Reference Substrates	Cellulose (%)	Xylan(%)	Surface Lingin Coverage(%)
KP0	82.20	16.78	Negligible
KP13	79.91	16.05	29.36
KP40	77.23	15.44	45.53
SP0	87.41	9.65	Negligible
SP13	85.65	9.52	16.47
SP40	82.87	9.21	39.23

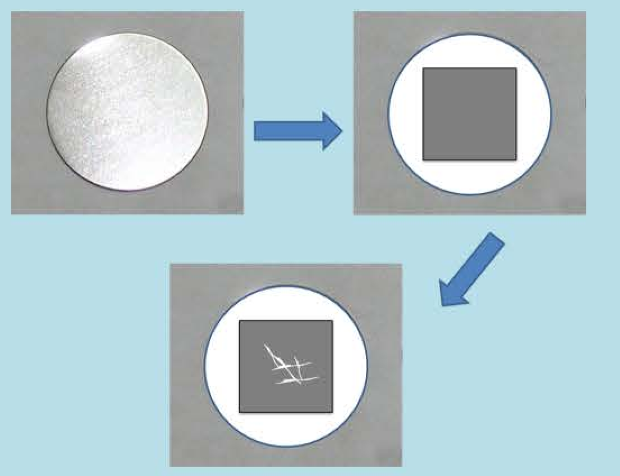


Figure 3. Silicon substrate fibril addition

Figure 4. Fibril samples

Conclusion & Future Work

We confirmed the fact that KP samples have stronger attractive forces with methyl group compared to SP samples. Higher hydrophobic interactions may increase the enzyme adsorption of the lignocellulosic biomass, which could result in improving enzymatic hydrolysis efficiency.

We will further quantify the morphological changes of those reference substrate surfaces along with the hydrophobic interaction forces.

References & Acknowledgement

(1) Wyman, C. E. (1996) *Handbook of bioethanol : production and utilization*, Taylor & Francis, [Bristol, PA]

(2) Zhang, Y., and Lynd, L. (2004) Toward an aggregated understanding of enzymatic hydrolysis of cellulose: Noncomplexed cellulase systems. *Biotechnology and Bioengineering* 88, 797-82

This work, as part of the Northwest Advanced Renewables Alliance (NARA), was funded by the Agriculture and Food Research Initiative Competitive Grant no. 2011-68005-30416 from the USDA National Institute of Food and Agriculture. In addition, we wish to acknowledge Deliorman, Muhammedin and Quisenberry, Chrystal Rae for their supportive help with AFM technique and patience.