

Automated quantitative cellular scale characterization of acid-pretreated poplar using confocal microscopy and image analysis

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The conversion of lignocellulose biomass (LB) as a natural renewable resource into bio-based products can be a solution to increasing concerns about climate change and increasing demand for energy. Nonetheless, LB is naturally recalcitrant to enzymatic deconstruction. To enhance the conversion of LB a pretreatment step is crucial to enhance enzymatic hydrolysis of LB. Dilute acid pretreatment is one of most efficient and widely used pretreatment methods [1]. Extensive research has been done to study the effect of dilute acid pretreatment on the cell wall composition and subsequently on the hydrolysis yield. Until now, little is known about the impact of pretreatment at cellular and tissular scale.

We used a combination of confocal imaging and quantitative image analysis to investigate the cellular scale impact of dilute acid pretreatment. 3D images of pretreated poplar samples were acquired using a confocal laser scanning microscope (Leica TCS SP8, Germany). We developed an automated 3D segmentation and quantification pipeline to process the acquired z-stacks. The images were first denoised using Alternating Sequential Filter (ASF) and Gaussian filters. To identify individual cell walls, the filtered z-stacks were then segmented using a 3D watershed algorithm whose seeds were determined using the h-minima operator which computed local minima regions in the denoised z-stacks. The 3D watershed algorithm provided a 3D image in which voxels (volumetric pixel) of the same cell were labeled by a unique integer. Thresholding was then used to compute cell walls by replacing the voxels of the segmented images with background value. Following 3D segmentation of z-stacks, individual cell wall volumes and cell wall surface area were computed (using voxel counting and marching cubes algorithms respectively) to determine sphericity, a dimensionless cellular scale parameter, for each individual cell. The results revealed a negative correlation between sphericity and pretreatment severity. Furthermore, cellular sphericity was also negatively correlated with hydrolysis yield establishing quantitative relationships across nano and micro scales [1].

[1] Zoghلامي, A., Refahi, Y., Terryn, C., & Paës, G. Multimodal characterization of acid-pretreated poplar reveals spectral and structural parameters strongly correlate with saccharification. *Bioresource Technology*, 293, 122015 (2019).