

TOPOCHEMISTRY, POROSITY AND CHEMICAL COMPOSITION DETERMINING SUCCESSFUL ENZYMATIC SACCHARIFICATION OF SUGARCANE BAGASSE

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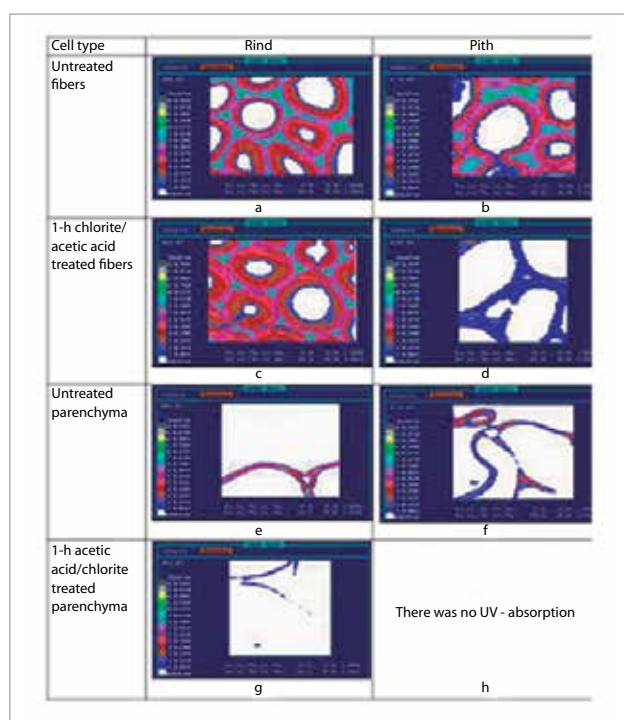


Figure 1. Scanning UV-micrographs of 1µm transverse sections of sugar cane cells. Appropriate software translates the absorptions intensities at 278 nm (shown in the left of the image) into multiple colors to illustrate the lignin distribution in the biomass tissues. The image clearly indicates the cell corners as the region with the highest absorption (colored with light- and dark green), followed by the middle lamella (colored with light green to pink) and by the secondary cell wall (colored with pink to dark blue) (Reproduced from Siqueira et al. 2011)

Lignocelulosic biomass is recalcitrant to enzymatic digestion because terrestrial plants develop an efficient manner to grow upward and resist the microbial degradation of the polysaccharides contained in their cell walls. The complex cell ultrastructure, varied tissues, and the composite characteristic of the cell walls are among the several factors explaining the recalcitrance of lignified plants. Mapping the macromolecular components in the cell walls has proved to be useful to understand the varied recalcitrance of different biomass tissues. Available data indicate that lignin and hemicellulose greatly affect the final digestibility of the lignocelulosic materials. Removal of these components from the cell walls with varied pretreatments or even using lignin- and/or hemicellulose-depleted plants indicate that a critical characteristic of the cell wall to be digestible is to present most as possible available cellulose. Saccharification of sugarcane bagasse based on the enzymatic hydrolysis of forthcoming plants down regulated on lignin biosynthesis or prepared by using a selective chemical step followed by mechanical fiberizing was developed at mild conditions. These bagasse samples served as models to find the desirable characteristics, mainly in terms of lignin content, lignin topochemistry and cell wall porosity, necessary to minimize the harshness or abolish the treatment that precedes the enzymatic hydrolysis of the available polysaccharides. Cellular ultraviolet microspectrophotometric evaluation of the samples

suggested that in the parenchyma and vessel cell walls, the hydroxycinnamic acids were linked to the lignocellulose backbone mainly through alkali-labile ester linkages. By contrast, the C-4 positions of the aromatic rings of the hydroxycinnamic acids contained in fiber cell walls should be etherified to lignin. The pretreatment caused intense delignification in the majority of the internode regions. The outermost regions were the most resistant to lignin and hemicellulose removal (Figure 1). Enzymatic hydrolysis of the pretreated samples indicated that the outermost fraction and the rind were recalcitrant regions, whereas the pith-rind interface was less recalcitrant.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

Sugarcane bagasse with different lignin contents were prepared by chlorite delignification providing more accessible cellulose, resulting in higher hydrolysis rates. More than 90 % of the cellulose was converted into glucose by removing 63 % of the initial lignin, while all cellulose was hydrolyzed after removing 72 % of the lignin. Combining the effect of lignin removal and the addition of β -glucosidases to the reaction medium, less lignin needed to be removed to achieve similar hydrolysis levels. The UV microspectrometry of lignin moieties in different cell types of mature sugarcane samples showed the highest UV absorbance in the cell walls of vessels followed by fibers and then parenchyma (Figure 2). Highly lignified fibers from the rind region of the internode become less recalcitrant as a function of the lignin and hydroxycinnamic acids removal. Untreated parenchyma cells from the pith region were promptly hydrolyzed by commercial cellulases indicating that the action of the cellulolytic enzymes was not restrained by the aromatics occurring in the pith parenchyma, but it was strongly controlled by the high lignin content present in the fiber cell walls from the rind region of the internode. The chlorite treatment led to significant removal of hydroxycinnamic acids and lignin of the rind cells in vascular bundles, resulting in a significant enhancement of the cellulose conversion by commercial cellulases. The total hemicellulose content in sugarcane bagasse increased from pith toward rind, therefore the sum of hemicellulose and lignin was a key factor to explain the varied recalcitrance of the different tissues. The alkaline/sulfite pretreated material was less recalcitrant to the enzymatic hydrolysis, since 85 % of cellulose conversion was obtained. The sulfonic acid groups turn the residual lignin less hydrophobic allowing an increased capacity of water retention by the fibers. The swollen fibers become more porous, facilitating the enzyme permeation toward the secondary walls of the pretreated material. The cellulolytic enzymes apparently do not adsorb irreversibly to the lignin in this type of pretreated material, which diminishes the enzyme load required for efficient hydrolysis as well as turn the enzymes recycling more feasible. These combined effects have been claimed to bring low enzyme consumption and costs in this type of process.

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