Sugarcane Cell Wall Structure and Degradation Investigated by Microscopy approaches

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Lignocellulosic biomass has been considered as potential source to second generation biofuel production. The technology used to conversion of fermentable sugar into bioethanol involves pretreatment, which is essential to the solubilization and separation of the complex lignin-hemicellulose-cellulose, making the remaining cellulose available to subsequent biological treatments. The plant cell wall molecular architecture remains unclear and it has been related to recalcitrance of biomass to deconstruction. In this work, we have applied microscopy approaches to obtain detailed information of sugarcane cell wall organization, as well as, analyze its deconstruction after termochemical pretreatment at molecular level. Lignin and cellulose distribution in sugarcane tissue was determined by incubation with Safranine fluorescent staining. Lignin is more concentrated in the vessel wall than to secondary wall in parenchyma cells. After pretreatment, while lignin level decrease, the cellulose is not affected. In cell wall, lignin distribution was also analyzed by transmission electron microscopy in ultrathin sections stained with 2% $KMnO_4$. TEM images clearly showed lignin distribution in secondary cell wall, middle lamella and transitional zone. In pretreated sample, abnormalities in cell wall structure were visualized. In this case, lignin was seen as dark droplets been translocated from cell wall. Lignin droplet formation by thermochemical pretreatment was also clearly observed by scanning electron microscopy. The distribution of the filaments array in sugarcane cell wall was evidenced by atomic force microscopy. Fibers were organized as polylaminated structure, with distinct orientation, where transverse orientation is predominant. The measured filaments thickness is about 20 nm, which correspond to cellulose macrofibril size. Also, we have observed that thermochemical pretreated PCW had undergone considerable loss of filaments, completely affecting the cell wall integrity. Our results give insights that may help to understand cell wall organization and pretreatment effectiveness, which is crucial in terms of research and technology to biofuel production.

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