The origin of recalcitrance in sugar cane hybrids with contrasting lignin contents and chemically pretreated materials

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The recalcitrance of lignocellulosic materials to enzymatic hydrolysis has been clearly recognized to be associated with the limited porosity of lignified cell walls. This limited porosity hinders enzyme infiltration into non-pretreated lignocellulosic materials. Lignin and hemicelluloses are the major components limiting enzyme infiltration into the cell walls. These components also encapsulate cellulose microfibrils, preventing the access of cellulases to the cellulose chains. Hydroxycinnamic acid content is also important for explaining recalcitrance in grasses. In such monocotyledons, the varied chemical compositions and structures of the individual cell types add complexity to the understanding of the recalcitrance in these plant materials. Plants engineered or selected for low lignin contents have been reported to be less recalcitrant for direct enzymatic digestion. In the particular case of sugar cane, recent results evaluating the enzymatic hydrolysis of transversal cuts from varied internode regions indicated that only the central region of the internode is suitable for direct enzymatic hydrolysis. This is the same region with low lignin content, confirming lignin as a key component related to the recalcitrance of the cell walls. In the present work, eleven sugar cane experimental hybrids, contrasting in their lignin and extractives contents, plus two reference materials were evaluated according to their chemical composition and digestibility by hydrolytic enzymes. Selected samples were also evaluated according to the topochemical distribution of lignin and hydroxycinnamic acids in the cell walls. The low hydrolysis levels observed for the untreated bagasse confirmed that the accessibility of enzymes into the fiber cell walls was very low. In most cases,

released glucose accounted for less than 20% of the available cellulose in the samples. In alkaline-sulfite pretreated materials, the enzymatic digestibility was proportional to the lignin removal caused by the pretreatment. Lignin sulfonation increased the hydrophilicity of alkali-sulfite pretreated substrates and may also contribute to promote the saccharification process. With the reference sugar cane material, the removal of approximately 50% of lignin and 30% of hemicelluloses by the pretreatment was enough to improve cellulose hydrolysis to 85% after 96h of hydrolysis. At shorter hydrolysis time (24h), 50% of the cellulose was hydrolyzed. The enzymatic hydrolysis of the experimental hybrid with the lowest initial lignin content (16.8 %) reached 64% cellulose conversion after the same hydrolysis period. Data from these studies show possible benefits for using samples with reduced initial lignin contents during the pretreatment and enzymatic hydrolysis processes. Therefore, determination of the topochemical distribution of lignin and aromatics in sugar cane was used to give relevant information on the varied recalcitrance of specific cells. Cellular UV-microspectrophotometry was used to topochemically detect lignin and hydroxycinnamic acids in cell walls of sugar cane. Vessels presented the most lignified cell walls, followed by fibers and parenchyma. Pith parenchyma cell walls were characterized by very low absorbance values at 278 nm. Untreated pith samples, which are rich in parenchyma cells, were promptly hydrolyzed by cellulases, reaching 81% of cellulose conversion after 72 h of hydrolysis. However, the pith region represented only 6% of the sugar cane dry biomass despite representing 11% of its total volume. By contrast, the untreated rind samples were hydrolyzed only to the limited level of 20%. This hydrolysis level was increased up to 90% only when approximately half of the initial lignin content was removed from the material by a chemical pre-treatment. An inverse correlation was observed between cellulose conversion levels and lignin contents or absorbance data for the varied tissue types in rind.

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