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

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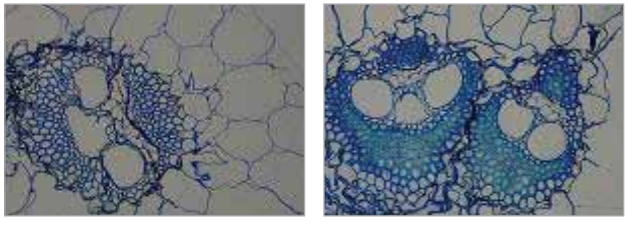

*Figure 1. Complexity of sugarcane anatomy. (A) pith; (B) rind*

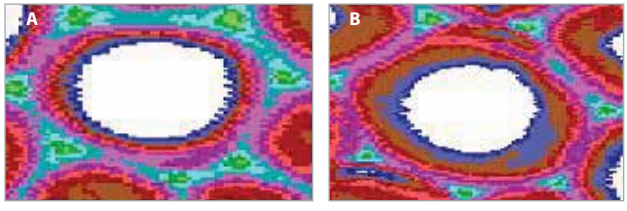


*Figure 2. Topochemical distribution of lignin in rind fibers of sugarcane (A) and 1-h chlorite delignified sugarcane (B). In these pictures, blue and brown colors means low lignin contents, while red-pink and light green colors means high lignin contents*

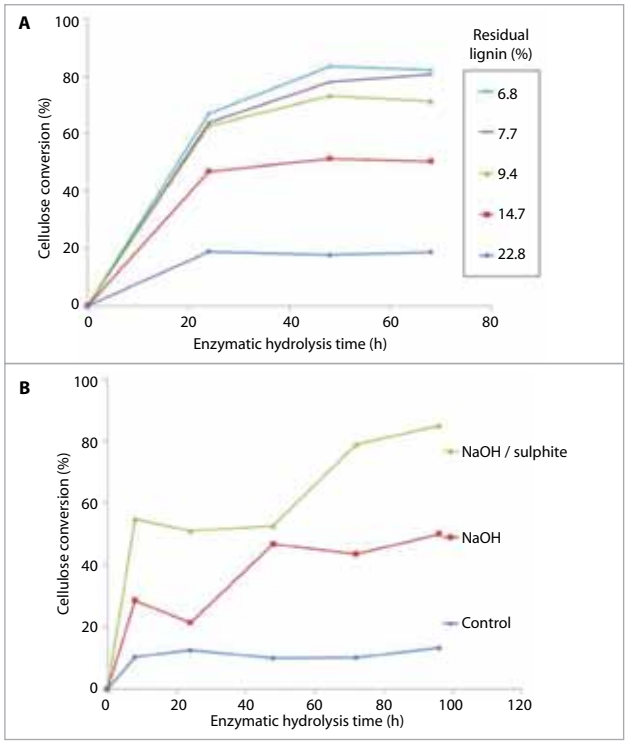


*Figure 3. Effect of chlorite treatment time (A) and alkaline and alkaline/sulphite chemothermomechanical pretreatment (B) on time-dependent cellulose conversion of bagasse*

This project proposes the development of a new concept for saccharification of sugarcane bagasse based on the enzymatic hydrolysis of forthcoming plants down regulated on lignin biosynthesis. The hypothesis is that once a sugarcane bagasse with reduced lignin content would be available, the hydrolysis of the entire polysaccharide fraction could be performed at mild conditions. Nowadays, the methods used in the pre-treatment of lignocellulosic materials, which precedes the advantageous enzymatic hydrolysis are quite harsh and inevitably lead to degradation and loss of valuable carbohydrates. Moreover, they are generally energy-intensive and generate undesirable by-products, which significantly add processing costs. To check on this hypothesis, the present proposal plan to prepare sugarcane bagasse samples with progressively reduced lignin contents by using a selective chemical step followed by mechanical fiberizing. These bagasse samples would serve 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, namely hemicellulose and cellulose. A subsequent step will be the evaluation of the plants with decreased lignin contents from studies involving down-regulation of lignin biosynthesis or from hybrids selected for low lignin contents.

## SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

Sugarcane cell anatomy is complex. Vessels are surrounded by fiber bundles. This cell groups are distributed along all internodes in the stalk. The inner part of the internodes named pith (*Figure 1A*) contains few of this cell groups and a large number of wide-thin-walled parenchyma cells. On the other hand, the outermost part, usually named rind, contains a large number of fiber bundles and smaller parenchyma cells (*Figure 1B*).

This morphological variation is relevant for all intents of sugarcane bagasse hydrolysis. Certainly, lignin and polysaccharide distribution varies in these different cell walls. Cellular UV-microspectrophotometry from untreated cell walls showed the presence of ferulic and p-coumaric acids linked to lignin or arabinoxylans. Vessels presented the most lignified cell walls followed by fibers and parenchyma. Pith parenchyma is not extensively lignified but contains significant amounts of ferulic and p-coumaric acids.

Cellular images showed highest lignin concentration in middle lamella and cell corners. One-hour chlorite treatment promoted rapid delignification and ferulic acid removal on parenchyma cell walls, while thicker fiber cell walls (*Figure 2A*) were only slightly delignified after 1-h treatment (*Figure 2B*).

Current breeding programs already provide sugarcane lines with lignin content as low as 16% compared to 25% in control plants. These plants are under study in the current project. However, first results have been obtained with chlorite delignified models and alkali-treated bagasse. Enzymatic hydrolysis of the entire sugarcane bagasse with 10 FPU Celluclast/g of dry material for 48 h resulted in the hydrolysis of 21.5% and 66.6% of the original cellulose in the untreated (22.8% lignin) and 2h-chlorite treated sugarcane bagasse (9.4% lignin), respectively (*Figure 3A*). Removal of 33% of lignin and 13% of hemicelluloses by NaOH pre-cooking improved saccharification levels to 50%. Alkaline-sulphite pre-cooking increased lignin and hemicellulose removal to 53% and 29%, respectively, reaching 85% saccharification after 96h of enzymatic hydrolysis (*Figure 3B*). Treated samples seem to simulate well the plants with reduced lignin content. In a subsequent step, sugarcane lines with reduced lignin content would be evaluated under similar hydrolysis conditions.

## MAIN PUBLICATIONS


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