

## PAPER

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## Sugarcane bagasse delignification with potassium hydroxide for enhanced enzymatic hydrolysis

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The optimization of an alkaline pretreatment process for the delignification of sugarcane bagasse (SCB) to enhance the subsequent enzymatic hydrolysis was performed according to the Doehlert uniform shell design. In this experimental design, the effect of two factors—potassium hydroxide (KOH) concentration and autoclaving time at 121 °C (1 atm)—on cellulose, hemicellulose, or the total polysaccharide and lignin content in SCB was evaluated. This response surface methodology revealed that KOH concentration is the factor that most influences the chemical characteristics of treated SCB (SCB<sub>t</sub>), with optimal conditions for the highest delignification being KOH in the range 5–10% (w/v) and an autoclaving time of 35 min, which provides an average of 97% total polysaccharides without inhibitor accumulation (furfural, 5-hydroxymethyl furfural) and ≤5% lignin. SCB<sub>t</sub> samples from two pretreatment conditions (KOH 3.25% – 13 min; KOH 10% – 35 min) were selected, based on the greatest delignification (70–74%) and polysaccharide availability (95–97%) after pretreatment, and further hydrolysed for fermentable sugar production. High sugar yields were obtained from both the pretreated samples (866 to 880 mg sugar per g biomass, respectively) in contrast with the 129 mg sugar per g raw biomass obtained from untreated SCB. These results demonstrate the effectiveness of KOH alkali pretreatments, which improves the overall digestibility of raw SCB polysaccharides from about 18% up to 91%. However, harsh alkali treatment (KOH 10%) is the most effective if the highest glucose/xylose ratio in the final sugar-rich hydrolysate is the aim. Hence, the use of sugar-rich hydrolysates obtained from SCB<sub>t</sub> as the carbon source for industrial purposes may provide a sustainable and economic solution for the production of bio-based added-value products, such as second generation (2G) bioethanol.

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### 1. Introduction

Sugarcane (*Saccharum officinarum* L.) is a perennial grass that predominantly grows in tropical and subtropical regions and is the world's largest crop, with Brazil the largest producer.<sup>1</sup> Sugarcane bagasse (SCB), a fibrous residue of cane stalks left over after the crushing and extraction of sugar, is the main Brazilian agroindustrial residue with 250–280 kg produced per ton of sugarcane processed. Currently, about 50% of the SCB is burned to generate power for alcohol distilleries and sugar mills, and a smaller portion is used as animal feed. However, a large amount of SCB is still discarded leading to environmental problems.<sup>2–4</sup> Moreover, aligned with an increasing global demand for ethanol fuel there is the prospect of increased sugarcane production resulting in an even greater amount of SCB.<sup>5,6</sup>

SCB is a lignocellulosic biomass containing significant amounts of carbohydrates (60–70%), mostly in the form of two polysaccharidic molecules, cellulose (33–45%) and hemicellulose (28–35%), and a polyphenolic macromolecule, lignin (20–30%).<sup>2–4</sup> Cellulose, the most abundant component, is a polymer consisting of long unbranched chains of D-glucose units linked by β(1 → 4)-glycosidic bonds. Cellulose has crystalline and amorphous regions, with the former the main reason for its recalcitrance to the hydrolytic process. The second predominant constituent is hemicellulose, an amorphous, complex, branched and heterogeneous polysaccharide network, based on pentoses, hexoses and sugar acids. Hemicellulose has variable composition according to its source, and in SCB it is composed of heteroxylans, with mostly xylose. A hemicellulose matrix can be chemically hydrolysed more easily than cellulose.<sup>2</sup> Lignin is a three-dimensional amorphous phenolic polymer, which holds the hemicellulose and cellulose fibres. It has a complex structure formed by the polymerization of aromatic alcohols that are resistant to enzymatic attack and degradation, and thus its content and distribution is recognized as the most important factor that determines the recalcitrant cell wall hydrolysis.<sup>7–9</sup>

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