



**ANAIS**

**I WORKSHOP DO PROJETO TEMÁTICO FAPESP**

**Proc.: 08/56246-0**

**BIOPROCESS SYSTEMS ENGINEERING (BSE) APPLIED TO  
THE PRODUCTION OF BIOETHANOL FROM SUGARCANE  
BAGASSE**

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**São Carlos - SP**

**REALIZAÇÃO**

**Departamento de Engenharia Química – UFSCar**

**Empresa Brasileira de Pesquisa Agropecuária - EMBRAPA**

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## APRESENTAÇÃO

Este “I Workshop do Projeto Temático” tem como principal objetivo a apresentação de propostas e de resultados obtidos durante o primeiro ano de desenvolvimento do Projeto Temático: **“Bioprocess Systems Engineering (BSE) Applied to the Production of Bioethanol from Sugarcane Bagasse”**, financiado pela Fundação de Amparo à Pesquisa do Estado de São Paulo – FAPESP (Processo 2008/56246-0), no bojo do programa FAPESP/PRONEX/BIOEN, com vigência de junho de 2009 a julho de 2013. O projeto, proposto conjuntamente pelo Departamento de Engenharia Química da UFSCar e pelo grupo de Bioprocessos da Embrapa Instrumentação Agropecuária, incorpora atualmente colaborações com outros laboratórios e instituições como Instituto de Catálisis y Petroleoquímica (Consejo Superior de Investigaciones Científicas, Espanha), Institute of Resource and Energy Technology (Technische Universität München, Alemanha), Programa de Engenharia Química da COPPE/UFRJ e do Grupo de Intensificação, Modelagem, Simulação, Controle e Otimização de Processos da UFRGS. O projeto é coordenado pelo Prof. Dr. Roberto de Campos Giordano.

O tema do projeto foi subdividido em **cinco subprojetos interligados**, que buscam promover o conhecimento aprofundado do tema e o desenvolvimento de tecnologia para a produção de bioetanol a partir de bagaço da cana-de-açúcar:

- a) Desenvolvimento, implementação e validação de um ambiente computacional integrado amigável, permitindo simulação, otimização, avaliação econômica, análise de CO<sub>2</sub>, análise de dados cinéticos e automação de biorreator para processos de produção de etanol lignocelulósico.
- b) Cultivos de microrganismos a partir do banco da Embrapa (*Aspergillus sp.*), para a produção de celulases e xilanases usando reatores trifásicos não convencionais, incluindo bagaço pré-tratado no meio.
- c) Pré-tratamento físico-químico do bagaço: explosão a vapor, remoção da hemicelulose e delignificação. Produção de substratos para rotas de produção de bioetanol via fermentação de hexoses.
- d) Determinação das condições (sub-)ótimas para a produção de etanol a partir da celulose.
- e) Avaliação da produção de etanol a partir da hemicelulose usando enzimas livres e imobilizadas.

## MICROESTRUTURAL COMPARISSON BETWEEN DIFFERENT PRETREATMENT METHODS IN SUGAR CANE BAGASSE

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The abundant lignocellulose which is a potential feedstock for 2<sup>nd</sup> generation biofuel production is made up predominantly of cellulose, hemicellulose, and lignin, fractions that must be hydrolyzed in order to produce fermentable sugars. Besides the composition and enzymatic mechanistic, there are numerous structural factors (e.g., crystallinity, available fiber surface area, pore structure) that lead enzymatic hydrolysis. All these need to be addressed for cellulosic ethanol to emerge on an industrial scale. Constraints in the large scale process summarize in three critical steps: pretreatment, enzymatic hydrolysis, and fermentation. Between them the pretreatment is cited as the first step if we are to achieve high yields from the subsequent biological operations.

In this context, sugarcane bagasse (SCB), abundant byproduct from Brazilian industry, was pretreated by acid, alkaline and combined methods with the principal objective to compare and evaluate its impact in its composition, microstructure and morphology. Factors like crystalline cellulose given by X-ray diffraction (DRX), structural composition by fourier transformed infrared spectroscopy (FTIR) and microscopic changes by scanning electron microscopy (SEM) will assist to elucidate the quality and potential of the treated SCB for enzymatic conversion.

For pretreatments (PT) method, a mass of 50 g of dried SCB was grinded to attain 1 mm size and then treated with solutions of NaOH, H<sub>2</sub>SO<sub>4</sub> at 2% (w/v) in a 1:5 (v/w) ratio. The PT were done in autoclave at 121°C for 30 min. For the combined pretreatment, SCB samples submersed in a H<sub>2</sub>SO<sub>4</sub> solution during 24 h, were abundantly washing with distilled water until pH 5, the complementary basic solution were carried out with the same parameters used in the autoclave reactor. After all the treatments, samples were washed with distilled water. Then the substrates were dried in an oven at 60 °C for 5 h.

The chemical composition was determined by AOAC methodology. In order to infer structural information, FTIR spectra was scanned in the range 4000 e 400 cm<sup>-1</sup> using a KBr disc containing 1% of each finely ground pretreated sample. The crystallinity index (CrI), which is correlated with the crystalline cellulose was calculated based on the method of Segal et al. (1959). Finally, morphological effects of the PT were monitored by SEM in (Au/Pt) coated samples.

After application of all the methodology and spectroscopic techniques, the results in Table 1 show the mass yield associated with each pretreatment, the chemical composition and the IC of the untreated and pretreated SCB.

All the PT caused a cellulose concentration due to the hemicellulose and lignin elimination. The final composition of lignin in alkaline PT sample indicates a remotion of 80%. As reported by literature the main effect of NaOH is delignification by breaking the ester-cross linking lignin, it resulted too in lesser cellulose and hemicellulose solubilization than acid and combined PT. On the other hand, the acid PT aims to solubilize hemicellulosic fraction in sugars (xylose, arabinose, etc.). A decrease of 92% in hemicellulose content after acid treatment confirms this statement. The combined PT derived in both reduction of lignin and hemicellulose (83% and 80%, respectively). To complement this information, CrI in Table 1 were calculated from DRX spectra (data not shown). As can be observed all PTs caused an increase in these values due to relative concentration of crystalline cellulose. The massive removal of amorphous components suggests the recalcitrant and crystalline nature of remaining cellulose.

Figure 1 shows untreated and pretreated SCB spectra by FTIR. The vertical lines in red, blue and green mark positions of the bands ascribed to hemicellulose, lignin and cellulose respectively (Zhao et al., 2008). The carbonyl band at 1735 cm<sup>-1</sup> representative to hemicellulose is reduced in all pretreated SCB. As well as, lignin bands at approximately 1595 and 1510 cm<sup>-1</sup> (aromatic ring stretch) are strongly reduced in the alkaline and acid/alkaline pretreated samples compared with both acid treated and untreated SCB.

Table 1. Relative chemical composition and crystallinity indexes of untreated and pretreated SCB.

Component (%)	SCB	Alkaline PT	Acid PT	Combined PT
Mass Yield	-	60,56	52	43,23
Extractives	2,44	-	-	
Cellulose	46,62	66,44	68,62	86,67
Hemicellulose	26,51	25,48	2,29	5,40
Lignin	21,7	4,42	25,98	3,67
Ash	2,51	1,87	1,22	1,99
IC	58,18	69,90	69,28	73,99

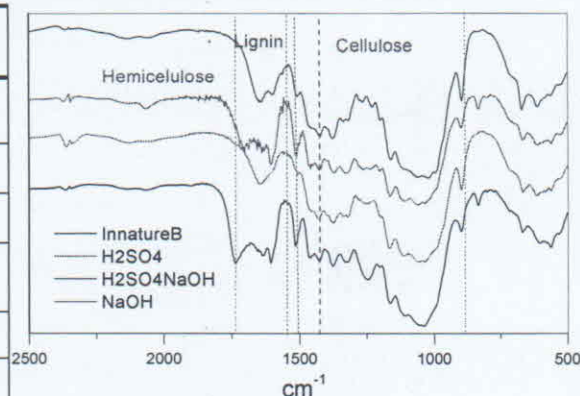


Figure 1. Untreated and PT SCB FTIR spectra.

Relative amounts of amorphous and crystalline cellulose have earlier been described through FTIR peak ratios at  $1429\text{ cm}^{-1}$  (crystalline) and  $893\text{ cm}^{-1}$  (amorphous) (Wistara et al., 1999). So the intensification in  $1429\text{ cm}^{-1}$  should correspond to the concentration of crystalline cellulose, according to the CrI in Table 1.

An entire comparison between all SEM micrographs (Figures 2A, 2B, 2C and 2D) reveals in general structural loosening of vegetal cells (epidermis and parenchyma tissue). Untreated SCB (Fig 2A) shows a disrupted structure derived from previous operations of milling and washing, original from sugarcane processing.

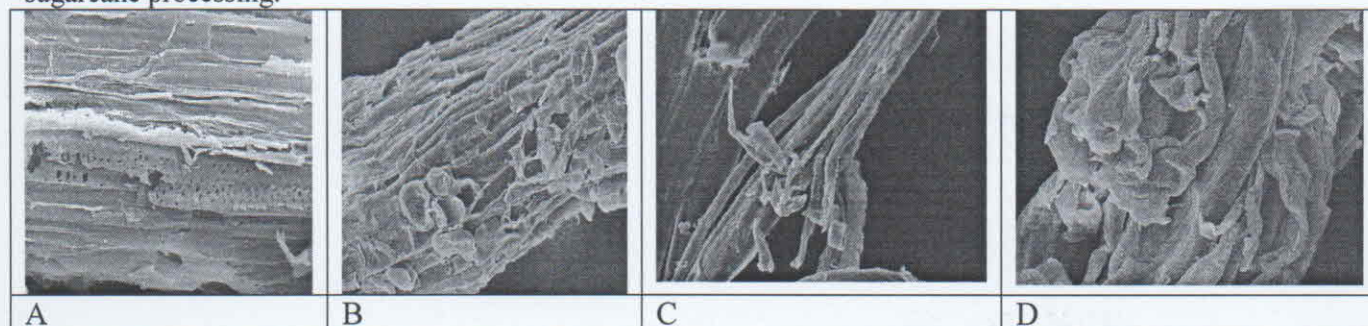


Figure 2. SEM micrographs of untreated and pretreated SCB. A) untreated SCB 500x. B) acid treated SCB 1000x. C) alkaline treated SCB 1000x and D) acid/alkaline treated SCB 2000x.

Fig 2B displays a partial defibrillation and the exposition of vascular bundles after acid pretreatment. Moreover, in relation to alkaline PT (Fig 2C), it can be observed a highlighted exposure of microfibrillar cellulose structure derived from the solubilization of lignin and hemicellulose. Finally the acid/alkaline PT (Fig 2D) promoted a partial removal of microfibrils and the appearance of amorphous cellulose aggregates.

Results show that although, partial hemicellulose and lignin removal is an important factor in increasing the digestibility of SCB as an enzymatic substrates, the PT and conditions used in this study yielded a cellulose of crystalline nature, unavailable for subsequent bio-ethanol application. Finally the use of rapid and non destructive methods like spectroscopic techniques is capable to monitor structural and physical changes after appropriated validation. Relevant information which allow anticipate and set up technical issues regarding the large scale design of of the biomass in order to achieve substantial progress.

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