

RESEARCH AND DEVELOPMENT AIMING AT THE INTEGRATED EXPLOITATION OF SUGARCANE BAGASSE FOR THE BIOTECHNOLOGICAL PRODUCTION OF LIGNOCELLULOSIC ETHANOL

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Figure 1. Growth of xylose-fermenting yeasts in bagasse fiber and fermentation of sugarcane bagasse hydrolysate in a batch bioreactor aimed at bioethanol production

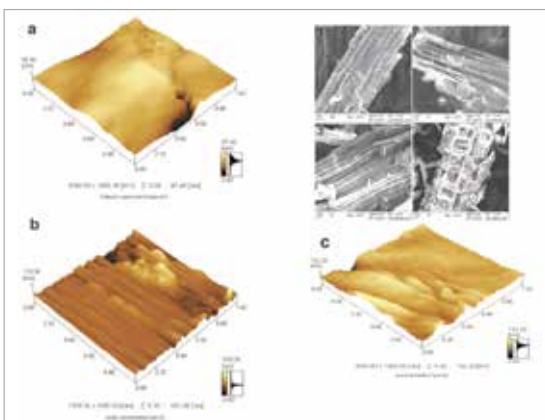


Figure 2. Atomic force microscopy (AFM) amplitude images. (a) Native sugarcane bagasse (b) Oxalic acid-pretreated bagasse (c) Enzyme hydrolysed bagasse and Scanning electron microscopic analysis of sugarcane bagasse. Show surface image of (1) natural, (2) dilute sulfuric acid pretreated, (3) sodium hydroxide pretreated cellulignin, and (4) enzyme hydrolyzed bagasse

The project aimed to accomplish the fractioning of sugarcane bagasse in its main components (cellulose, hemicellulose and lignin) for their use in the production of ethanol.

Acid hydrolysis was used to remove the hemicellulosic fraction followed by alkaline hydrolysis. Enzymatic hydrolysis of the cellulose fraction was then performed. After each stage of fractioning, the solids were characterized by advanced spectroscopic techniques including Raman scattering, infrared absorption with Fourier transformation (FTIR), absorption in the near infrared ray (NIR), thermal lens analysis and photo-acoustic analysis. The use of these techniques was interesting because of their nondestructive character, possibility of *in situ* measurements and potential for further development of compact prototypes. Next, the obtained xylose- and glucose-rich hydrolysates were properly treated through chemical, physical and biological detoxification methods and were used as fermentation media for the production of ethanol by several new xylose-fermenting yeasts and by *S. cerevisiae*, respectively. Xylose-arabinose fermenting species isolated from the Atlantic Rain Forest, Amazon Forest and Brazilian Cerrado ecosystems were tested. Yeasts inhabiting rotting-wood substrates were collected and tested for xylose-arabinose fermentation. This project also aimed to find new species from these Brazilian ecosystems capable of being used in industrial processes.

In all of the unitary operations involved in this project, conditions were optimized by experimental design and data analysis by means of appropriate statistical methodologies. The obtained results allowed the establishment of innovations and advanced technologies that stand to extend the national and international competitiveness of second-generation "bioenergy" technologies for Brazil's national alcohol program. They also allowed the formation of teams and encouraged cooperation among the participant institutions for training and exchange of knowledge.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

This project involved the use of different pretreatments using dilute sulfuric acid, oxalic acid fiber expansion and concentrated aqueous ammonia soaking, which revealed marked hemicellulose degradation and lignin removal from sugarcane bagasse, thus improving the accessibility of cellulases and releasing fermentable sugars from the pretreated substrate. The acid-base pretreatment process was scaled up to 350-L and helped identify potential problems associated with scale-up prior to investing in expensive full-scale equipment.

Structural studies based on spectroscopic principles for characterization of sugarcane bagasse after sequential acid-base pretreatments and enzymatic hydrolysis showed marked changes in hemicellulose and lignin removal at the molecular level.

New yeast species able to utilize pentoses as carbon sources for ethanol production were screened and identified by physiological and molecular methods, and some of these yeasts presented cellulolytic activity.

The cellulosic material showed high saccharification efficiency after enzymatic hydrolysis. Hemicellulosic and cellulosic hydrolysates revealed ethanol production by xylose-fermenting yeasts and *S. cerevisiae* under batch fermentation conditions.

Complementary studies on the influence of agitation, aeration and initial pH were made to optimize ethanol productivity in a stirred-tank bioreactor under controlled conditions. Cell immobilization techniques were presented as a method for improving ethanol production, in this case, a basket-type stirred tank bioreactor and fluidized bed bioreactor that were used to optimize xylose-to-ethanol bioconversion.

The project also demonstrated that electromagnetic field bioreactors present a new and promising research area for improvement of yield and/or productivity in the fermentation process. An electromagnetically fluidized bed bioreactor with cells immobilized in magnetic particles was tested, and the performance of ethanol production of hemicellulosic hydrolysate was affected by the electromagnetic field configuration and intensity. In conclusion, this research generated scientific knowledge regarding methods and perspectives related to the integrated use of sugarcane bagasse for bioethanol obtainment.

MAIN PUBLICATIONS

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