ETHANOL PRODUCTION FROM SUGARCANE BAGASSE: ENZYMATIC HYDROLYSIS, MICROBIOLOGICAL ASSAYS TO EVALUATE TOLERANCE OF YEASTS TO THE TOXICITY OF HYDROLYSATES AND FERMENTATION AT HIGH TEMPERATURES

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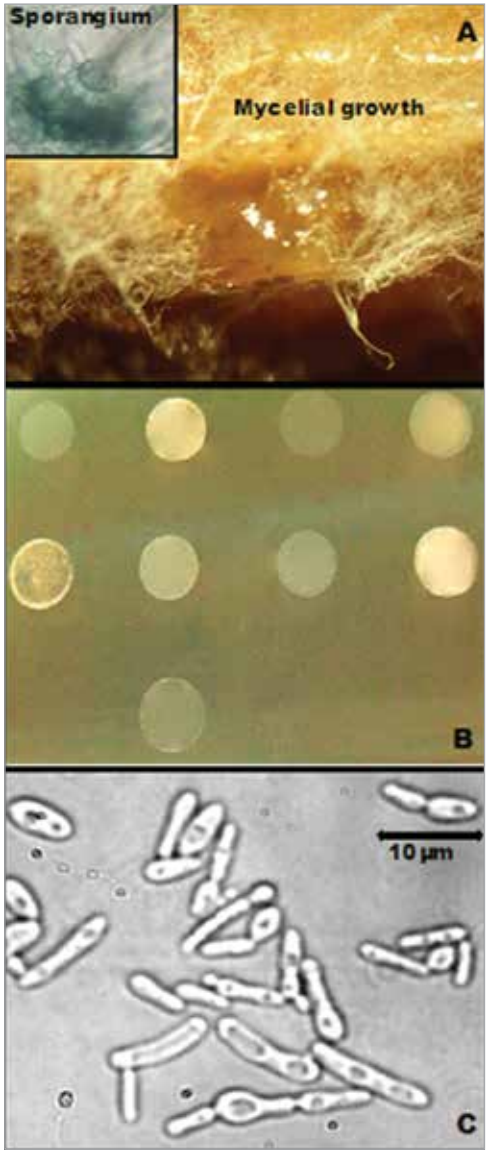

Figure 1. (A) a sporangium (phase contrast microscopy, 1000Í) and the mycelia growth (phase-contrast stereoscopy, 35Í) resulting from the propagation of Aspergillus nidulans *on sugarcane bagasse; (B) Selection of yeasts strains based on growth on plates containing high concentration of both sugar and acetic acid; (C) cells of* I. orientalis *able to convert glucose into ethanol in simple batch cultures at 42°C*

The hydrolysis of cellulolytic materials with diluted acids is well known, but this process generates toxic products of hydrolysis. Other negatives factors related to the acid hydrolysis are the corrosion and the high amounts of salts resulting from the acid neutralization. The production of enzymatic preparations at lower cost showing activity at lower pHs and resistance to its reuse are needed. In addition, the fermentation of cellulolytic hydrolysates depends on the yeast strain and the levels of toxic compounds present in the hydrolysates.

Physicochemical methods reported in literature for the pre-treatment and hydrolysis of bagasse as well as the use of crude preparations of cellulolytic enzymes produced by fungi will be evaluated and improved. The identification and quantification of the activity of each enzyme of the enzymatic complex able to hydrolyze the sugar-cane bagasse will be another target of this investigation. The production of ethanol by simultaneous saccharification and fermentation (SSF) of sugar-cane bagasse will be also studied.

There is a great need for the development of fast and reliable microbiological methods to assay yeasts strains and levels of the toxicity of the hydrolysates. Frequently, the circumstances preceding the arrest of the fermentation and types of changes of the fermentation profiles can provide valuable information. Assays have to be developed to predict how the fermentation will proceed. A synthetic medium will be optimized and used as a reference medium to study the effects of inhibitors produced during the bagasse hydrolysis and their interactions with respect to growth and fermentation using statistical methods. This medium will be used as a tool in fast diagnostic assays to evaluate the toxicity of the hydrolysates and the tolerance of the yeast strains to acidity and levels of inhibitors prior to the fermentation process. Solid media will be developed for the qualitative evaluation the toxic inhibitors of hydrolysis on the yeast growth capacity.

As temperatures greater than 30°C-34°C are observed in industrial reactors operating in tropical countries, the search for yeasts strains tolerant to acidity and high temperatures are required for hydrolysates fermentation. Strains tolerant to acidity and temperature will be used in the present study. Temperature usually aggravates the effects of other stress determinant factors. Assays in bioreactors will allow the optimization of the entire process for maximal efficiency of the ethanol production.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

Among several fungi studied, *Aspergillus nidulans* was seen in the present work as the most promising fungi for the production of cellulolytic enzymes when grown on sugarcane bagasse pretreated with diluted acid at room temperature. The production of the CMCase was high in the culture inoculated with mycelia, while the avicelase showed greater activity in the culture containing spores. However, the same total cellulose activity was obtained from both mycelia and the spores cultures. The results obtained in the present work indicated that the filtrate from the *A. nidulans* cultures could be used as a crude cellulolytic preparation for saccharification of sugarcane bagasse and also as a medium for the ethanol production for containing high levels of total reducing sugar and low amounts of phenol.

Solid and liquid media were modified to study the effects of sugarcane bagasse inhibitors on yeast growth and fermentation. Some yeast strains were able to growth on plates containing high concentration of sugar and acetic acid, while growth of other strains was inhibited. Using a synthetic medium containing 18% glucose (w/v), the effects of increasing concentrations of acetic acid were evaluated. At this high sugar concentration, the growth decreased when the levels of the acetic acid increased up to 58 mM . Above this concentration, a low but constant biomass was obtained up to 330mM acetic acid added to the reference medium. The effects of the formic acid and furfural on ethanol secretion and growth were much greater than those obtained with acetic acid, while levulinic acid showed a lesser effect on growth and fermentation.

Issatchenkia orientalis is a non-Saccahromyces yeast able to convert simple sugars into ethanol at high temperatures and low pH values. Strains of this yeast were isolated from cultures growing at temperatures ≥ 38°C. An amount of 7.0 % ethanol (v/v) was obtained when 10% glucose was fermented by this yeast at 42°C in YPD medium. The same amount of ethanol was obtained when molasses containing 10% total reducing sugar was fermented for 12 hours at 42°C in a co-culture of *S. cerevisiae* and *I. orientalis*. This co-culture was inoculated to obtain an initial biomass concentration of 10 g. L^{-1}. Thus, it seems possible to use this yeast to ferment hydrolisates of sugarcane bagasse when added to molasses or sugarcane syrups.

MAIN PUBLICATIONS

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