TITLE: ENZYMATIC DETOXIFICATION OF HEMICELLULOSIC HYDROLYZATE FROM SUGARCANE BAGASSE FOR PENTOSE FERMENTATION AND ETHANOL PRODUCTION

Authors: [1,2] Tramontina, R.; [1,2] Franco Cairo, J.,P.; [1] Santos S., C.; [1,2] Sousa, A.,S.; [1] Ruller, R; [1] Lenczac, J.,L.; [1] Squina, F.; [1] Damásio [1,2] A.,R.,L.

Institution [1] Laboratório Nacional de Ciência e Tecnologia do Bioetanol - CTBE/CNPEM, Campinas, São Paulo, Brazil. [2] Universidade estadual de Campinas – UNICAMP, Campinas, São Paulo, Brazil

Abstract:

Plant cell wall polysaccharides are high potential sustainable resources for biotechnology. The conversions of these polymers into fermentable sugars are a key challenge into second generation fuels' chain. Related to that, after the thermal pre-treatment of sugarcane bagasse for enzymatic saccharification, as a residue a hemicellulolytic hydrolyzate (liquor C5), which is rich in pentose, furfurals and lignin is obtained. However, due the presence of toxic compounds and fermentation inhibitors in C5 liquor, even *Scheffersomyces stipitis* which stands as a key microorganism that utilizes xylose for ethanol production has unsatisfactory fermentation levels on it. Related to that, the identification and characterization of genes and enzymes involved in lignocellulosic materials are essential to the economic viability in biorefinery. The aim of this work was the in-situ fermentation inhibitors present in the C5 liquor elimination, and increase levels of ethanol produced by *S. stipitis*. A recombinant enzyme from the termite (*Coptotermes gestroi),* which is involved in redox metabolism processes in biomass degradation (CgREDOX-1) was produced and characterized for this purpose. The methodology proceeded with CgREDOX-1 amplification, cloning and expression in Arctic express (*Escheria coli* expression strain).

The expressed enzyme was purified by affinity and gel filtration chromatography. The production CgREDOX1 resulted in 15 mg/l culture of functional protein, which was physico-chemically characterized. C5 liquor was buffered to pH 5.7 and 5mg / l of CgREDOX-1 and 0.02 mM NADPH were added, the incubation time was carried out for 16 hours at 30 degrees. Furfurals and xylose concentration were measured by HPLC. Moreover, enzyme assays have shown that a number of aromatic compounds are reduced by the enzyme. C5 liquor treated and untreated with enzyme will be conducted with alcoholic fermentation by *S. stipitis* strain in complex medium containing liquor C5 to 33% for 96 hours. Production of ethanol and xylitol will be evaluated by HPLC and the yeast growth rate will be measured in a spectrophotometer at 600nm. It is expected that the treatment do not decrease the concentrations of fermentable sugars and degrade furfurals in C5 liquor.

Key words: *Coptotermes gestroi*; Bioethanol; *Scheffersomyces stipitis*; enzymes; recombinant proteins.

Grant: FAPESP