In Situ cellulase production for 2nd generation bioethanol

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Production and use of cellulolytic enzyme complex (CEC) are key factor to viability of bioethanol from lignocellulose material. The cost of available commercial CEC preparation is one of the highest of this technology. In order to minimize this cost it has being proposed the production and use of CEC at the same site of bioethanol production: the so-called i*n situ* CEC production. We have been studying bagasse and other agricultural residues as carbon source aiming the *in situ* CEC production in submerged fermentation using filamentous fungi isolates. *Trichoderma harzianum* strain best results of filter paper (FPase), xylanase and β-glucosidase activities were attained with bagasse treated by steam explosion (EB), bagasse treated by steam explosion followed sodium hydroxide delignification (EDB) and hydrothermic treated bagasse (HB) using Mendel culture media at 29°C in shaker flasks and lab scale bioreactor, attained, respectively, up to 1,000 FPU/L, 30,000 IU/L and 2,000 IU/L, complains 1 g/L of active enzyme protein in 72h. Culture media supplementation with sucrose, wheat flour and soybean flour enhanced about 40% FPase activity. Also it was observed a sharply increase up to 10 times for xylanase and β-glucosidase activities with the tested supplementation probable due wheat and soybean flour composition and enhancement of biomass growth due to sucrose presence in culture media. Initial experiments with fed batch cultivation mode leads us to anticipate additional increment towards the bench mark of 10 g/L active enzyme protein in culture broth. The centrifuged and concentrated culture broth was used to perform hydrolysis of pretreated bagasse (10% w/v; 50°C, pH 4.8) and the data were compared with *Trichoderma reesei* RUT C30 supernatant and commercial enzyme preparation, attained up to 50-60% in terms of glucose yield based on pretreated bagasse composition. Recombinant cellulases prepared in our laboratory are also being tested to supplement the produced fungi enzymes.

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