Environmental Engineering and Management Journal March 2012, Vol.11, No. 3, Supplement, S94
http://omicron.ch.tuiasi.ro/EEMJ/

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PRODUCTION OF HOLLOCELLULOLYTIC ENZYMES USING AGRO-INDUSTRIAL RESIDUES: SELECTION OF TYPE OF PRETREATMENT AND SUBSTRATE

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Abstract

Biofuels of second generation are produced using agro-industrial residues which are complex materials composed mainly of cellulose, hemicellulose and lignin. The aim of this work was to evaluate the acid and alkaline-pretreatment of sugarcane bagasse, agave bagasse and corn cob in terms of physical structure changes, lignin removal, production and activity of cellulases and xylanases (commonly denominated holocellulases) for the mutant PR-22 of *Cellulomonas flavigena.*

A factorial experimental design of three factors was carried out: substrate at three levels (agave bagasse [AB], sugarcane bagasse [SCB] and corn cob [CC]); pretreatment at two levels (acid and alkali) and concentration at three levels (low [L], medium [M] and high [H]). Main experimental variables were volumetric activities of CMCase and xylanases, among others. AB, SCB and CC were pretreated in acid and alkaline dilute solutions (1-3% v/v or w/v). The effects of each pretreatment were also evaluated by scanning electron microscope (SEM) images and lignin removal. *Cellulomonas flavigena* mutant PR-22 was used for hollocellulase production. Experiments were carried out at 37 °C, pH 7 and 150 rpm in 125 mL shake flasks.

Comparing with acid-pretreatment, alkaline-pretreatment was positive for hollocellulases production for all substrates with 23 and 53% higher volumetric xylanolytic and cellulolytic enzymatic activities, respectively. Xylanase activities were higher in substrates treated with the highest concentration of alkali; however, in these conditions CMCase activities were not improved. Concerning the type of substrate, CC had the best inducer effect of hollocellulolytic enzymes ($p < 1x10^{-4}$).

Alkaline-pretreatment removed among 20-50% of lignin content of residues, whereas acid pretreatment did not remove lignin at all. Moreover, SEM images showed that alkaline pretreatment was more effective in separating and thinning the fibers.

Alkaline pretreatment was more suitable for lignin removal from the lignocellulosic fibers and led to higher activities of holocellulases.

CC gave higher hollocellulase activities of *C. flavigena* PR-22 compared to agave and sugarcane bagasses. Our results indicate that use of alkali-pretreated CC and post fermented by *C. flavigena* mutant PR-22 is a promising substrate to obtain high titers of hollocellulases.

Key words: agro-industrial residues, *Cellulomonas flagivena*, holocellulases, pretreatment