

Nattawadee Jantasila 2012: Investigation on Suitable Pretreated Agricultural By-Products for Cellulase Production and Regulation of Cellulase Expression. Doctor of Philosophy (Biotechnology), Major Field: Biotechnology, Department of Biotechnology. Thesis Advisor: Associate Professor Sunee Nitisinprasert, D.Sc. 150 pages.

Three cheap agricultural waste sources in Thailand of rough rice bran (RRB), palm kernel meal (PKM) and cassava pulp (CP) containing lignocellulose were aimed to investigate the suitable pretreatment for production of cellulolytic enzymes from *Aspergillus niger* 386017M1 by solid state fermentation process (SSF). Comparing three different pretreatments of acid (AC), alkaline (AKL) and steam techniques (STE), the suitable pretreatment of RRB and PKM were STE providing the increasing cellobiohydrolase (CBH) activities of 6,244 and 1,626 units/g, and beta-glucosidase (BG) activities of 28,485 and 26,869 units/g, respectively. While the AC pretreatment was suitable for CP having CBH and BG activities of 7,303 and 9,349 units/g, respectively. The maximum growth of *A. niger* in solid state fermentation of both the control and suitable pretreated RRB, PKM and CP were 5 day with the pH of around 4–5. Comparing to the control, the CBH activities from SSF of STE-pretreated-PKM and AC-pretreated-CP significantly increased to 135 – 163 and 453 – 698 folds, respectively while the one of STE-pretreated-RBB was not significantly different. By chemical analysis, the lignin contents of STE-pretreated-RBB, STE-pretreated-PKM and AC-pretreated-CP were mostly reduced for 1.5, 1.4 and 1.3 folds of the control while the cellulose contents increased for 2.0, 3.2 and 1.6, respectively. This was emphasized by the Scanning Electron Microscope that showed the increasing of rough surface, open cell wall structure in all pretreated materials.

The acid pretreated CP was chosen to study in cellulase gene expression. The maximum CBH activities of 7,599 units/g substrates and growth of 113 ng/g were obtained from 5 d cultivation at pH 3.5. The expression of *cbhA* and *cbhB* were regulated by *xlnR*, *creA*, *pacC* and *ace2* which were measured at transcription level by qRT-PCR. Interestingly, the strong expression of *cbhB* was due to low catabolic repression of *creA* while the one of *cbhA* was to high expression of *xlnR* and *ace2*. The *pacC* gene repressed the alkaline-expressed gene of fungal during acid pH of SSF system. Obviously, activator elements of galactose and arabinose played a key important role in expression of *cbhB* to improve CBH production. This result allows direct studies of the novel evolution of cellulase mechanisms control and provides opportunities to obtain a broader perspective on the logic of regulatory circuits of *A. niger* on acid pretreated cassava waste.

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Thesis Advisor's signature