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Bioethanol production thanks to immobilized cellulolytic enzymes



March 19th, 2018, Karthik Periyasamy defended his doctoral thesis of the University Grenoble Alpes – prepared under the supervision of the Professors Marc Aurousseau and Gérard Mortha and the co-supervision of Agnès Boyer, Associate Professor (Grenoble INP-Pagora/LGP2) – and of the Anna University (Chennai, India), under the supervision of Professor Sivanesan Subramanian. He presented the results of his research work entitled ***Bioethanol production from lignocellulosic biomass using immobilized cellulolytic enzymes.***

The overall objective of the study was to produce bioethanol from lignocellulosic biomass by using free and immobilized xylanase, cellulase and β -1, 3-glucanase.

Specifically, this study was focused on the isolation of *Trichoderma citrinoviride* strain AUKAR04 able to produce xylanase (55,000 IU/gds), cellulase (385 IU/gds) and β -1, 3-glucanase (695 IU/gds) in solid state fermentation. Then the free enzymes were biochemically characterized depend on effect of pH, temperature and metal ion concentration and corresponding kinetic parameters were determined. Then the enzymes were subjected to two types of immobilization using carrier-free co-immobilization (combi-CLEAs) method or bifunctionalized magnetic nanoparticles (ISN-CLEAs) with higher thermal stability, extended reusability and good storage stability.

Liquid ammonia pretreatment removed 40% of lignin from the biomass and retained 95% of glucan, 65% of xylan and 41% of arabinan in sugarcane bagasse (SCB). SCB was enzymatically hydrolyzed and converted to 87 % glucose from cellulose and 74% of xylose,

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64% of arabinose from the hemicelluloses which is remarkably higher than the activity of the free enzymes.

Chemical and structural analysis of SCB was done by ATR-FTIR, TGA and XRD. FTIR result showed a successful pretreatment of the SCB raw material. It showed that hemicelluloses and cellulose are partially depolymerized by the action of xylanase, cellulase and β -1,3-glucanase in ISN-CLEAs. TGA studies showed that the thermal stability of the ammonia pretreated and enzymatically treated samples have improved remarkably. XRD results showed that the crystallinity index of the ISN-CLEAs treated SCB increased to $61.3 \pm 1\%$ when compared to the ammonia-treated SCB. Mono-culture fermentation using *Saccharomyces cerevisiae* LGP2Y1 utilized SCB hydrolysate containing 103.8 g/L of glucose and produced 42 g/L ethanol in 36 h of fermentation. The overall metabolic yield achieved was about 79% of theoretical yield. Co-culture fermentation using *Saccharomyces cerevisiae* LGP2Y1 and *Candida utilis* ATCC 22023 utilized SCB hydrolysate containing 107.6 g/L of glucose and 41.5 g/L xylose and produced 65 g/L ethanol in 42 h of fermentation. The overall metabolic yield in co-culture fermentation achieved was about 88 % of the theoretical yield.

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