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# KINETIC AND ENZYME RECYCLING STUDIES OF IMMOBILIZED β-GLUCOSIDASE FOR LIGNOCELLULOSIC BIOMASS HYDROLYSIS

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## Abstract

β-glucosidases are very important in the improvement of cellulose degradation rates by diminishing the inhibition of end products. Although, cellulase cost has reduced significantly in last decade but still the high costs of biomass saccharification is one of the main challenges for the commercialization of ethanol from LC biomass. In this work, β-glucosidase immobilization in calcium alginate was optimized using response surface methodology. 89% immobilization efficiency was achieved under optimum conditions (i.e., calcium alginate 4.19% (w/v), calcium chloride 0.14 M and β -glucosidase 4.42% (v/v) or 57.46 U/mL of reaction mixture). pH and temperature optima of both free and immobilized β-glucosidase was found to be 5 and 50 °C, respectively. Enzyme kinetics of free and immobilized β-glucosidase was done to evaluate Michaelis constant (Km) and Vmax values, using p-nitrophenyl-β-D-glucopyranoside (pNPG) as the substrate and glucose as inhibitor. Increase in Km value (0.995 mM) for immobilized β-glucosidase as compared to the free β-glucosidase (0.617 mM) was observed. A decrease in Vmax from (1191.74 μmol $min^{-1}$ for free β-glucosidase) to (736.65 μmol $min^{-1}$ immobilized β-glucosidase) was observed after immobilization. Glucose was found to inhibit β-glucosidase by competitive mode of inhibition (Ki= 3.01 mM). Studies were carried out in a batch reactor using 2% cellobiose, 0.1 M sodium acetate buffer, 5 pH and 50 °C. 84% cellobiose to glucose conversion was found in first cycle which was about 67% in fifth cycle. This study suggests that enzyme immobilization improved enzyme stability, substrate inhibition and permits enzyme reusability.

*Key words:* alginate, biofuel, cellulose, immobilization, kinetics

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