Biosynthesis of Xylitol from Sugarcane Bagasse: Process Optimization, Modelling and Intensification

A

Thesis

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THESIS ABSTRACT

In the first part of this thesis, statistical optimization of process parameters for dilute acid hydrolysis of sugarcane bagasse for maximum xylose yield was reported. Prior to statistical optimization, the chemical composition of sugarcane bagasse and the effect of solid to liquid ratio on xylose yield were analysed. The process parameters considered for optimization were hydrolysis temperature, acid concentration (or acid load) and hydrolysis time. Optimum levels of these parameters were determined by Box Behnken design (BBD) method of optimization. The optimum values of the process parameters for dilute acid hydrolysis of sugarcane bagasse were investigated. The composition of monomeric sugars (xylose, glucose, and arabinose) and inhibitory products (acetic acid, furfural, and 5–HMF) in the hydrolysate has also been analyzed under optimal conditions of dilute sulfuric acid hydrolysis of sugarcane bagasse. In subsequent investigations, we addressed the kinetic and thermodynamic features of dilute acid (2% v/v H_2SO_4, 1:30 w/v) hydrolysis of sugarcane bagasse. Time profiles of xylose formation in range of 100°–130°C and treatment period of 0–120 min were analysed with modified biphasic Saeman model. Generation of glucose, arabinose and inhibitory products (furfural, 5–HMF and acetic acid) were also analysed. Easy–to–hydrolyse fraction of hemicellulose increased with temperature. Activation energies for hydrolysis and xylose degradation were investigated. Thermodynamic analysis (ΔH, ΔS, and ΔG) revealed that xylose formation is thermodynamically more favoured than degradation.

In the second part of the thesis, optimization of medium components and process parameters for xylitol production from sugarcane bagasse using *C. tropicalis* MTCC 184 immobilized on PU foam were investigated. Plackett–Burman design revealed that out of seven medium components, 4 medium components, viz. yeast extract, $MgSO_4 \cdot 7H_2O$, KH_2PO_4 and $(NH_4)_2SO_4$; as significant components. These medium components were further optimized using central composite design (CCD) method to find their optimum values. Optimization of process parameters, viz. temperature, initial pH, shaking speed and substrate (xylose) concentration, was done at the optimized values of medium components. Optimum values of these parameters for maximum xylitol yield = 0.65 g/g of xylose are: yeast extract = 5.78 g/L, $(NH_4)_2SO_4$ = 3.22 g/L, KH_2PO_4 = 0.58 g/L, $MgSO_4 \cdot 7H_2O$ = 0.57 g/L and temperature = 29.3^{o}C, initial pH = 6.2, shaking speed = 151 rpm and initial xylose concentration = 20.9 g/L. Medium components provide essential growth factors and utilizable potassium, phosphate,

nitrogen, sulphur sources. Activity of xylose reductase in metabolic pathway is stabilized and augmented by Mg^{2+}.

In the third part, we addressed ultrasound–assisted xylitol production through fermentation of dilute acid (pentose–rich) hydrolysate of sugarcane bagasse using free and immobilized cells of *Candida tropicalis*. Sonication of fermentation mixture at optimum conditions (optimized in the second part) was carried out in ultrasound bath (37 kHz and 10% duty cycle). Time profiles of substrate and product in control (mechanical shaking) and test (mechanical shaking + sonication) fermentations were fitted to kinetic model using Genetic Algorithm (GA) optimization. Maximum xylitol yield of 0.56 g/g and 0.61 g/g of xylose was achieved in control and test fermentations, respectively, in free cells of *C. tropicalis*. Moreover, the xylitol yield of 0.65 g/g and 0.66 g/g of xylose was obtained in control and test experiments, respectively in immobilized cells of *C. tropicalis,* which essentially corresponded to 71% and 73% of the theoretical yield.

Although sonication of fermentation mixture resulted in marginal (17–20%) rise in final xylitol and biomass yields, the kinetics of the fermentation showed drastic enhacement. 2.5× enhancement in xylitol productivity and 2× rise in specific uptake rate of xylose was achieved under ultrasound assisted fermentation. Comparative assessment of the parameters of kinetic model for fermentation under test and control condition revealed that sonication promoted the uptake and utilization of substrates for cell growth and increased enzyme–substrate affinity. The inhibition effect of substrate was also reduced with sonication. 10–20× enhancement in permeability of cell membrane caused faster diffusion of substrates, nutrients and metabolite products across the cell membrane, which resulted in faster xylose metabolism and enhanced kinetics of fermentation. Flow cytometry analysis of in control (mechanical shaking) and test (ultrasound–treated) fermentations was carried out. The results of flow cytometry essentially confirmed that SSC and FSC of the *C. tropicalis* cells remain practically unaltered after sonication. Thus, the internal complexity and morphology of the cells remain unchanged after exposure to ultrasound, or in other words, no noticeable adverse impact of sonication is seen on the yeast cells.