



Abstract

Bioconversion of different pre-treated agricultural feed stocks into saccharides was carried out using thermophilic cellulases. Agricultural by-products *i.e.* sugarcane bagasse, wheat and rice straw were subjected to acid and alkali at steaming temperatures, auto-hydrolysis and microwave pre-treatments. Auto-hydrolysed and alkali pre-treated substrates showed better delignification *i.e.* 86.01% (wheat straw), 77.84% (sugarcane bagasse), 67.23% (rice straw) vs. 84.11% (wheat straw), 73.90% (sugarcane bagasse), 68.47% (rice straw), respectively. These de-lignified substrates were analyzed for total phenolic content (TPC) which were subsequently removed by washing with distilled water, extraction by organic solvents and detoxification by calcium hydroxide. Minimum residual TPC was observed in auto-hydrolysed substrates using calcium hydroxide *i.e.* 52 mg gallic acid equivalent (GAE) per g of dry weight (DW) of substrate in wheat straw, 59 mg GAE/g DW in sugarcane bagasse and 54 mg GAE/g DW in rice straw. The surface area *i.e.* 4 and 2mm granule size of all auto-hydrolysed substrates was assessed for saccharification potential and granule size of 2mm was found best with saccharification of 11.01% (sugarcane bagasse), 9.34% (wheat straw) and 4.36% (rice straw). Saccharification of auto-hydrolysed substrates using simultaneous addition of cellulases gave maximum saccharification in wheat straw *i.e.* 22.93% after 5 h of incubation at 80°C employing sodium citrate buffer of pH 6.5. Cellulase concentration of Endo-1,4- β -glucanase (125 U), Exo-1,4- β -glucanase (150 U) and β -1,4- Glucosidase (50 U) was optimized for 2% of wheat straw yielding 36.78% saccharification. Sequential addition of cellulases showed an improved saccharification of 55.64% employing Endo-1,4- β -glucanase (75 U), Exo-1,4- β -glucanase (100 U) and β -1,4-glucosidase (50U) after 5.5 h of incubation. Scale up of the sequential saccharification in a 50 L reaction vessel resulted in increased saccharification of 57.91% with decreased time of incubation (3.5 h). In addition, substrate concentration of 2.5 % with agitation of 100 rpm was optimized with consequent saccharification of 62.12%. After saccharification, the hydrolysate was analyzed for TPC which were removed using activated charcoal. Minimum TPC *i.e.* 64.43 mg GAE/mL of liquid phase was achieved using 3% activated charcoal at 40°C



after 20 min of incubation. The hydrolysate was analyzed by thin layer chromatography and found to have glucose and cellobiose. Wheat straw before auto-hydrolysis, after auto-hydrolysis and enzymatic breakdown was assessed for structural variance by scanning electron microscopy.