ABSTRACT

Agriculture and forest are considered to be the major sectors producing a large quantity of surplus residues annually. Exploitation of these surplus residues through efficient conversion technologies in terms of transportation fuels generation such as ethanol and diesel can reshape the energy scenario. The surplus residues are collectively called as lignocellulosic biomass which is composed of lignin, cellulose and hemicellulose. Lignin is the major recalcitrant molecule forming a barrier around the cellulose and hemicellulose molecules and limiting their access by cellulolytic enzymes for reducing sugars generation. Thus, the removal or breakdown of lignin is a pre-requisite to access the cellulose and hemicellulose molecules, which envisages the requirement of suitable biomass delignification process. Until now research has been concentrated towards removal or degradation of lignin through physical, chemical and physico-chemical methods followed by ethanol generation. These methods are associated with harmful fermentation inhibitors generation, hemicellulose solubilisation, hydrolysate neutralization and thus, polluting the environment.

The novelty in the present work lies on the application of tailor made enzyme for lignin degradation that does not produce any fermentation inhibitors, causes minimal hemicellulose solubilisation and no hydrolysate neutralization was needed as it operates under mild process environment without any chemical agents. The lignin degradation followed by ethanol production processes adopted in the present study are eco-friendly and sustainable in nature. A grass species called *Saccharum spontaneum* (Kans or Sarkanda grass) was used as a lignocellulosic feedstock for delignification, saccharification and fermentation that have the capability to grow and colonize the land quickly. It contains high holocellulose (~ 67 %, w/w) that makes it as a potential candidate for bioethanol production. Laccase obtained from *Lentinus squarrosulus* MR13 has been used for lignin degradation which has the capability to degrade the phenolic as well as non-phenolic subunits in the absence of synthetic mediators.

Optimization of various influencing process parameters for enzymatic delignification of Kans grass resulted in maximum lignin degradation of 81.67 % in 6 h. Saccharification or hydrolysis of delignified biomass was carried out using cellulase-xylanase cocktail produced from *Trichoderma reesei* Rut C30. Maximum reducing sugars of 500.30 mg/g was recorded in 5.30 h upon optimization. Various biomass processing technologies namely, simultaneous saccharification and fermentation (SSF), partial simultaneous saccharification and

fermentation (P-SSF) and consolidated bioprocessing (CBP) were adopted in the present work. Optimization of different process parameters of SSF, P-SSF and CBP resulted in maximum ethanol concentration of 5.34 % (v/v), 8.02 % (v/v) and 7.65 % (v/v) respectively. Scaling up at 5 kg and 25 kg batches through CBP resulted in 7.7 % (v/v) and 7.95 % (v/v) ethanol respectively. Structural characterization and biomass porosity analysis after each stage further substantiates the efficacy of the enzymes used for deconstruction and saccharification process. Thus, the results obtained in the present study supports the feasibility of the enzymatic venture towards eco-friendly second generation ethanol production from Kans grass.

Keywords: Lignocellulosic, Lignin, Holocellulose, Reducing sugars, Fermentation, Ethanol