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Green Bioethanol Production from Delignified Rice Straw Using Statistically Designed Crude Recombinant Enzyme Cocktail

Premeshworii Devi Maibam [a*] and Arun Goyal [a,b]
[a]*School of Energy Sciences and Engineering,* [b]*Carbohydrate Enzyme Biotechnology Laboratory, Department of Biosciences and Bioengineering, Indian Institute of Technology Guwahati, India.*
*p.maibam@iitg.ac.in (*Corresponding Author*)

Abstract

Around 60% of world's bioethanol production is from food and sugar crops, thereby giving rise to food vs. fuel conflict. Thus, focus has been shifted to second generation lignocellulosic bioethanol. The three crucial steps in the lignocellulosic biomass based biorefineries for bioethanol production are: i) pretreatment ii) enzymatic saccharification and iii) fermentation. However, the success in lignocellulosic biorefineries is evaluated upon overcoming the challenges associated with each step. The challenges are: i) development of green, recyclable and efficient pretreatment method, ii) designing of enzyme cocktail with optimum proportion of each component enzymes and iii) effective fermentation techniques for maximum conversion of fermentable sugars to bioethanol. To address these challenges, this study aims to i) exploit the potential of choline chloride-based deep eutectic solvent pretreatment method and optimise the process using rice-straw, ii) formulation of enzyme cocktail with optimum proportion of each constituting enzymes, crude recombinant clostridial cellulases (bifunctional chimeric enzyme, *Ct*GH1-L1-*Ct*GH5-F194A and cellobiohydrolase, *Ct*CBH5A) and xylanases (endo-1,4-β-xylanase, *Ct*GH11A and β-xylosidase, *Bo*GH43 from *Bacteroides ovatus*) for producing high yield total reducing sugars and iii) optimization of simultaneous saccharification and fermentation process parameters for maximizing ethanol titre. In this study, deep eutectic solvent comprising choline chloride (ChCl) and acetic acid (AA) was used for rice-straw (RS) pretreatment. The effect of molar ratio of ChCl:AA, time and temperature on lignin removal and total carbohydrate content (TCC) were evaluated by central composite design (CCD) approach. The optimum conditions for RS pretreatment were 1:3.6 (ChCl:AA molar ratio), 126°C and 2.5 h. ChCl:AA pretreated RS (CApRS) gave 83% delignification, 82% hemicellulose removal, 84% pretreatment efficiency and 679 mg/g_{CApRS} TCC. CApRS contained enriched cellulose content, 0.73 g/g_{CApRS}, 31% higher crystallinity index and 17-fold higher surface area than raw RS. For, the enzymatic saccharification of CApRS, the optimal proportion of crude recombinant enzyme cocktail was statistically designed by D-optimal mixture design approach. The developed enzyme cocktail comprised chimera, *Ct*CBH5A, *Ct*GH11 and *Bo*GH43 in ratio 35:42:10:13 giving cellulase activity, 5.6 FPU/mL. Enzymatic saccharification of 3%, w/v CApRS using developed enzyme cocktail at pH 5.7 (0.05 M sodium-phosphate buffer), 35°C and 187 FPU/g_{CApRS} yielded total reducing sugar (498 mg/$g_{biomass}$), glucose (410 mg/g) and xylose (71.3 mg/g) resulting in 72% saccharification efficiency. The developed enzyme cocktail gave a comparable performance to commercial enzyme cocktails and prospected its applicability in lignocellulosic biorefineries. Simultaneous saccharification and fermentation of CApRS using a developed enzyme cocktail and *Saccharomyces cerevisiae* NCIM 3215 was performed. Enzymatic hydrolysis of 10%, w/v CApRS at 35°C, pH 5.7 with 60 FPU/ g_{CApRS} of formulated enzyme cocktail for 100 ml was performed for 18h. After 18h of pre-hydrolysis, 5%, v/v of *S. cerevisiae* cells (10 OD_{600nm}) was inoculated and SSF in 100 ml at 30°C, 150 rpm was carried out. The SSF at constant pH of 5.7 with the feeding of an enzyme, 60 FPU/ g_{CApRS} at 6h and 12h, respectively amounting to total dosage of 180 FPU/g_{CApRS} resulted in a maximum ethanol titre of 25 g/L at 24h of fermentation and ethanol conversion efficiency of 75%.

***Keywords*:** Rice-straw, deep eutectic solvent, delignification, recombinant enzyme, enzyme cocktail**,** saccharification efficiency, simultaneous saccharification and fermentation, ethanol conversion rate.