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Formulation of a Novel Tailor-Made Enzyme Cocktail Playing a Synergistic Role in the Production of Fermentable Sugar from Pretreated Elephant Grass

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Abstract

Enzymatic saccharification plays a pivotal role in lignocellulosic bioethanol production. This process demands an extensive array of cellulolytic, xylanolytic and auxiliary enzymes for the complete hydrolysis of any lignocellulosic biomass. In order to obtain efficient hydrolysis, development of a tailor-made enzyme cocktail consisting of an optimized enzyme ratio is very crucial. In the current study, a novel tailor-made enzyme cocktail having synergistic activity of recombinant hydrolytic enzymes has been developed for the efficient hydrolysis of alkaline hydrogen peroxide pretreated elephant grass (AHPpEG). The cocktail comprised crude recombinant cellulases, xylanases and auxiliary enzymes. The recombinant cellulases used were bifunctional chimera, consisting of endo-1,4-β-endoglucanase and β-glucosidase (*Ct*GH1-L1-*Ct*GH5-F194A) and cellobiohydrolase (*Ct*CBH5A) from *Clostridium thermocellum*. The recombinant xylanases were endo-1,4-β-xylanase (*Ct*GH11A) from *Clostridium thermocellum* and β-xylosidase (*Bo*GH43) from *Bacteroides ovatus*, whereas the auxiliary enzymes used in this study was arabinofuranosidase (*Ps*GH43) from *Pseudopedobacter saltans*. The synergism among the recombinant hydrolytic enzymes was studied using different combinations of the component enzymes with a total enzyme dose of 50 U/mL. To perform the fine-tuning of the proportions of individual enzymes within the enzyme cocktail, a D-optimal design of experiment (DoE) was employed. This approach was utilized to design an enzyme cocktail with the aim to maximize the overall yield of TRS. The statistically optimized ratio of crude recombinant hydrolytic enzymes (*Ct*GH1-L1-*Ct*GH5-F194A: *Ct*CBH5A: *Ct*GH11A: *Bo*GH43: *Ps*GH43) was 43:20:10.8:10:16.2 giving an overall cocktail enzyme activity of 6.4 FPU/mL for cellulase using 50 mM citrate phosphate buffer, pH 5.4 at 35°C. The designed enzyme cocktail in 50 mM citrate phosphate buffer, pH 5.4 on incubation at 35°C displayed stability by retaining 61.3% and 53% residual cellulase activity over 24 and 48 h, respectively. The enzymatic hydrolysis of 2.5% (w/v) of AHPpEG in 50 mM citrate phosphate buffer, pH 5.4 by the developed enzyme cocktail at the total enzyme dose of 50 U/mL of pretreated biomass in 3 mL volume carried out at 35°C yielded TRS of 479 mg/g of AHPpEG. The study also explores the effect of four different surfactants (Na-cholate, Benzalkonium chloride, Triton X 100 and Tween 80) in the process of hydrolysis. The presence of 2% (v/v) Triton X 100 increases the TRS yield to 19% and results in 544 mg/g $_{AHPpEG}$ of TRS with the total glucose and xylose titre of 91.5 g/L and 25.2 g/L, respectively. The developed crude enzyme cocktail gave 75.5% saccharification efficiency highlighting its applicability in lignocellulosic biomass-based biorefineries.

***Keywords*:** Elephant grass, alkaline hydrogen peroxide, crude recombinant enzyme, synergism, total reducing sugar, enzyme cocktail**,** saccharification efficiency.