



## Optimisation of synergistic biomass-degrading enzyme systems for efficient rice straw hydrolysis using an experimental mixture design

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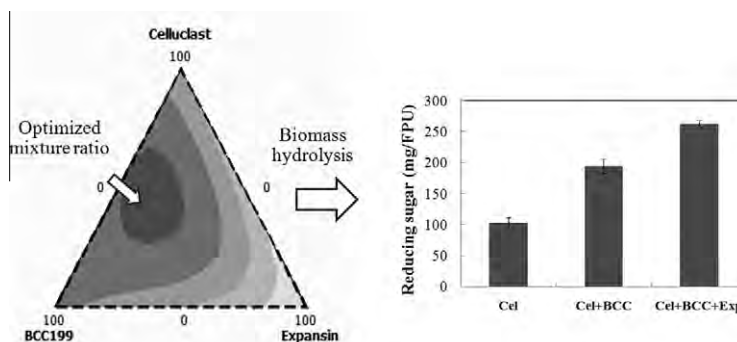
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### HIGHLIGHTS

- ▶ Mixture design was used for optimisation of ternary enzyme complex.
- ▶ Ternary enzyme system contained Celluclast™, *A. aculeatus* enzyme, and expansin.
- ▶ Synergy of expansin at practical cellulase dosage for biomass hydrolysis was shown.
- ▶ Proteomic profiles of crude enzymes from *T. reesei* and *A. aculeatus* were explored.
- ▶ This work demonstrated development of enzyme formulation for biorefinery industry.

### GRAPHICAL ABSTRACT



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### ABSTRACT

Synergistic enzyme system for the hydrolysis of alkali-pretreated rice straw was optimised based on the synergy of crude fungal enzyme extracts with a commercial cellulase (Celluclast™). Among 13 enzyme extracts, the enzyme preparation from *Aspergillus aculeatus* BCC 199 exhibited the highest level of synergy with Celluclast™. This synergy was based on the complementary cellulolytic and hemicellulolytic activities of the BCC 199 enzyme extract. A mixture design was used to optimise the ternary enzyme complex based on the synergistic enzyme mixture with *Bacillus subtilis* expansin. Using the full cubic model, the optimal formulation of the enzyme mixture was predicted to the percentage of Celluclast™: BCC 199: expansin = 41.4:37.0:21.6, which produced 769 mg reducing sugar/g biomass using 2.82 FPU/g enzymes. This work demonstrated the use of a systematic approach for the design and optimisation of a synergistic enzyme mixture of fungal enzymes and expansin for lignocellulosic degradation.

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## 1. Introduction

Lignocellulosic plant biomass represents the most abundant renewable carbon source and provides the basis for the sustainable biorefinery industry. Enzymatic saccharification of agricultural residues has long been envisioned as a promising approach for

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