

## **An optimal blend of single auto displayed cellulases for cellulose saccharification – a proof of concept**

### **ABSTRACT**

**BACKGROUND:** To date, it has been challenging to determine the optimal proportions of the individual cellulases in crude extracts and multienzyme systems to promote enzyme synergism. The application of single surface-display of enzymes may obviate such challenges. This article presents a proof of concept of the optimal mixing of single surface-displayed cellulases for cellulose hydrolysis using Simplex lattice mixture design.

**RESULTS:** The recently discovered maximized autotransporter-mediated expression system was used to express the three cellulases. The biochemical screening analysis revealed that the enzymes have a broad range of functional pH (4–9) and temperature (30–100 °C) characteristics. The evaluated optima were pH 6 and 60 °C. Through the statistical design of experiment, a blend ratio of 1: 1.6: 1.4 of endoglucanase: exoglucanase:  $\beta$ -glucosidase was identified as the 'sweet spot' for optimum sugar yield. The application of this blend generated about 0.354 mg mL<sup>-1</sup> and 0.446 mg mL<sup>-1</sup> of sugars from filter paper ( $\varnothing$  6 mm, ~2.5 mg) and 2.5% pretreated EFB, respectively, within 12 h. Supplementing the enzyme blend with CaCl<sub>2</sub> and Tween 20 improved the sugar yield.

**CONCLUSION:** This research has revealed an interesting concept of efficiently addressing cellulase synergism. The overall outcome of this research is promising for the derivation of value from lignocellulose.