

## **Optimizing Enzyme Cocktails for Hydrolysis of Mildly Pretreated Lignocellulosic Biomass**

Giuliano Dragone, Eva Balaguer Moya, Berta Syhler, Solange I. Mussatto\*

*Department of Biotechnology and Biomedicine, Technical University of Denmark, Søtofts Plads, Building 223, 2800, Kongens Lyngby, Denmark*

Commercially available cellulase cocktails often exhibit high efficiency in hydrolyzing easily digestible pretreated biomass, which typically lacks hemicellulose and/or lignin fractions. However, a challenge arises when it comes to the enzymatic hydrolysis of mildly pretreated lignocellulosic biomasses that contain significant amounts of cellulose, hemicellulose, and lignin. This study aimed to address this issue by evaluating the supplementation of a commercial cellulolytic cocktail with accessory hemicellulases and two additives (H<sub>2</sub>O<sub>2</sub> and Tween® 80).

Statistical optimization methods were employed to enhance the release of glucose and xylose from mildly pretreated sugarcane bagasse. The optimized supplement composition (417 µL g<sup>-1</sup> DM of hemicellulase, 65 µM of H<sub>2</sub>O<sub>2</sub>, and 521 mg g<sup>-1</sup> of Tween® 80) led to a production of 304 and 124 mg g<sup>-1</sup> DM of glucose and xylose, respectively. This represented a substantial increase of 84% in glucose release and 94% in xylose release compared to using only the cellulolytic cocktail (35 FPU/g).

The results obtained in this study using the combination of three supplements were also compared to the addition of only hemicellulase and only Tween® 80 to the cellulolytic cocktail. A significant increase in glucose release of 39% and 41%, respectively, was observed with the optimized combination. Similarly, for xylose, the increase was 38% and 41%, respectively.

This study underscores the substantial potential of optimizing enzyme cocktails for the hydrolysis of mildly pretreated lignocellulosic biomass through the use of tailored enzyme and additive combinations based on the specific composition of the biomass.