

## **Manipulation of nanofiber-based $\beta$ -galactosidase nanoenvironment for enhancement of galacto-oligosaccharide production**

### **Abstract**

The nanoenvironment of nanobiocatalysts, such as local hydrophobicity, pH and charge density, plays a significant role in optimizing the enzymatic selectivity and specificity. In this study, *Kluyveromyces lactis*  $\beta$ -galactosidase (Gal) was assembled onto polystyrene nanofibers (PSNFs) to form PSNF-Gal nanobiocatalysts. We proposed that local hydrophobicity on the nanofiber surface could expel water molecules so that the transgalactosylation would be preferable over hydrolysis during the bioconversion of lactose, thus improve the galacto-oligosaccharides (GOS) yield. PSNFs were fabricated by electro-spinning and the operational parameters were optimized to obtain the nanofibers with uniform size and ordered alignment. The resulting nanofibers were functionalized for enzyme immobilization through a chemical oxidation method. The functionalized PSNF improved the enzyme adsorption capacity up to 3100 mg/g nanofiber as well as enhanced the enzyme stability with 80% of its original activity. Importantly, the functionalized PSNF-Gal significantly improved the GOS yield and the production rate was up to 110 g/l/h in comparison with 37 g/l/h by free  $\beta$ -galactosidase. Our research findings demonstrate that the localized nanoenvironment of the PSNF-Gal nanobiocatalysts favour transgalactosylation over hydrolysis in lactose bioconversion.