ULTRAFILTRATION-BASED ENZYME RECOVERY IN THE BATCH PRODUCTION OF GALACTO-OLIGOSACCHARIDES

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INTRODUCTION

Galacto-oligosaccarides (GOS)-containing foods are becoming increasingly popular due to its prebiotic properties. At industrial-scale, GOS is produced from lactose by enzymatic synthesis in batch fashion using stirred-tank reactors (STR). Due to its high purchase price, the biocatalyst is considered as a major contributor to the overall raw material costs. Once the transgalactosylation reaction is completed, the enzymes are inactivated by heat treatment and removed from the resulting products by downstream operations.

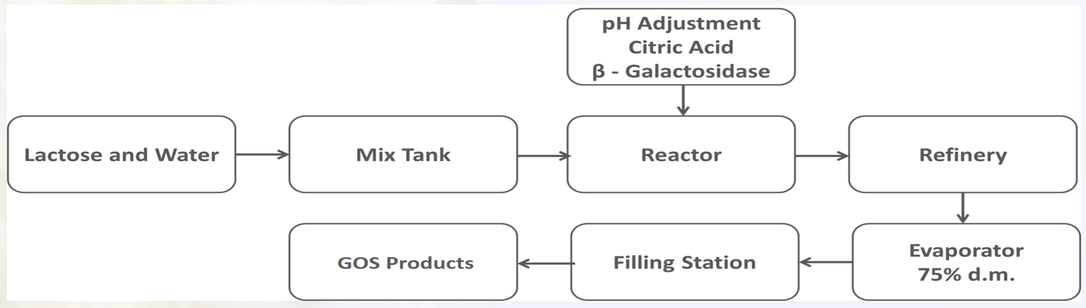

Figure 1. Scheme of process steps involved in the industrial production of galacto-oligosacchrides (Kovács et al., 2013).

RESULTS

Catalytic performance： **The decrease in the reaction velocity shows enzyme activity losses over the subsequent cycles.**

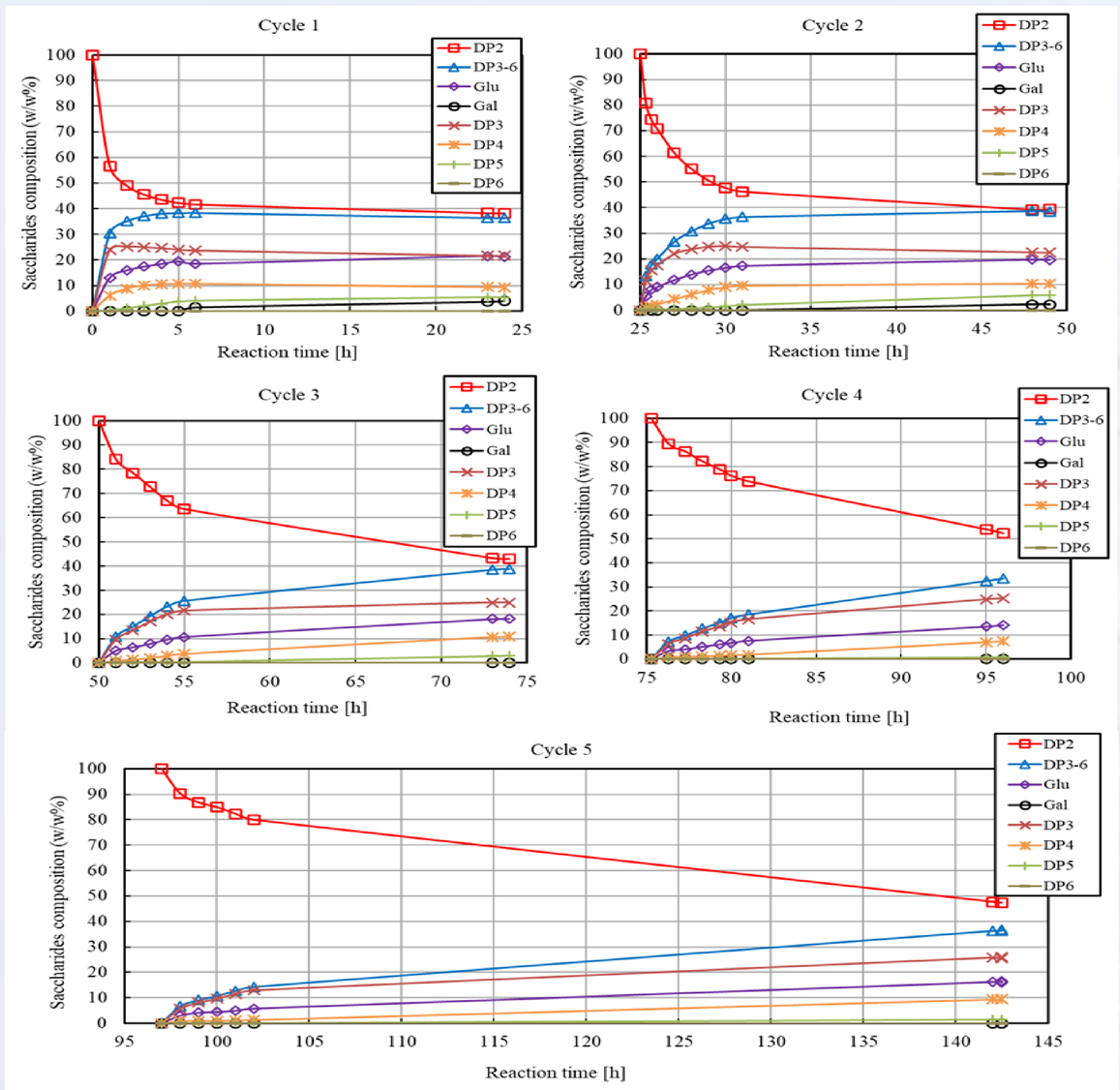

Figure 3. Saccharide's composition in the reactor as function of reaction time for the five consecutives) cycles.

Ultrafiltration performance：

- **The mean specific flux during UF concentration steps was measured as ca. 75 L/(h m2 bar) (30 kDa hollow-fiber PES module, FB02-CC-FUS0382STANDARD, Microdyn-Nadir, Germany).**
- **Membrane regeneration was successful using NaOH cleaning solution (pH=9, 40 degC, 0.1-0.2 bar, 1.5 h) as monitored by water permeability tests between cycles.**

Quantification of enzyme losses：

We consider a saturation model to describe the catalytic process:

$$F(t) = s*(1-\exp(-v*time))$$

where t is the reaction time, and s*v is the slop of the curve at t = 0.

Then, we use the initial reaction rate (s*v) values obtained from STR at known enzyme loads to generate a calibration model (Fig. 4) to estimate the remaining enzyme activity after each cycles.

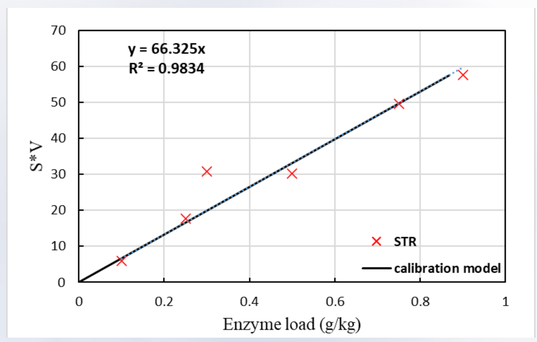

Figure 4. Linear model of s*v of STR as function of enzyme load.

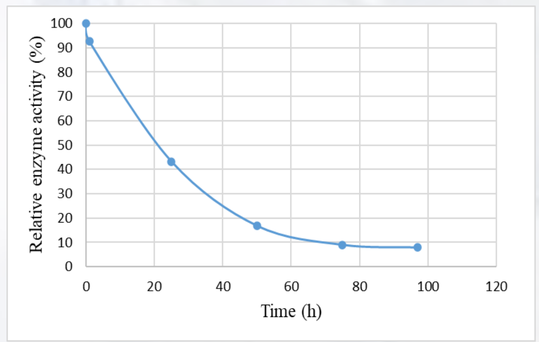

Figure 5. Relative enzyme activity as function of time in cyclic UF-assisted enzymatic membrane reactor.

The objective of our work is to investigate the feasibility of recycling the enzymes by ultrafiltration (UF) and reusing them in multiple cycles of transgalactosylation reactions.

AIM

MATERIALS AND METHODS

Laboratory experiments were carried out by following a protocol involving 3 operational steps.

- **In the first step, a soluble β-galactosidase of *Bacillus circulans* origin was used to convert lactose with a concentration of 30 w/w% into GOS in a conventional STR operating under fixed conditions of pH 6.0 and 50℃.**
- **In the second step, the reaction liquor was ultrafiltered to obtain an enzyme-free permeate consisting of the reaction products.**
- **Finally, a fresh substrate solution was added to the UF concentrate.**

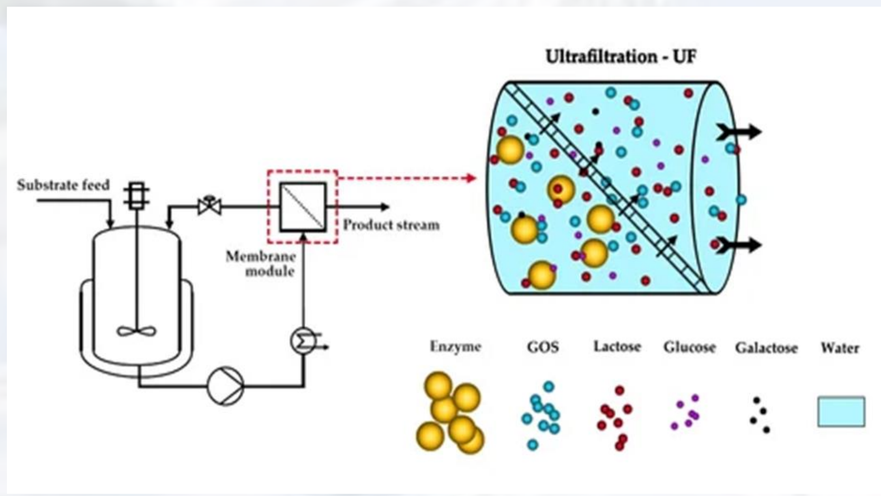

Figure 2. Schematic drawing of the cyclic ultrafiltration-assisted enzymatic membrane reactor.

This 3-step procedure was repeated in 5 consecutive cycles. The progress of GOS formation during the reaction in each cycle was monitored by HPLC, and enzyme activity losses were quantified by correlating the observed conversion rates with those measured in a series of STR tests with varying enzyme loads.

After UF (Step 2), the membrane was regenerated as follows:

- **the module was drained and flashed several times with DI water；**
- **membrane cleaning was carried out by circulating a NaOH solution (pH=9-10) for ca. 1-2 hour at 40-50 ℃ under 0.1-0.2 bar pressure；**
- **the module was drained and flushed several times with water to remove the cleaning agent；**
- **finally, permeability of the cleaned membrane was measured with DI water.**

CONCLUSION

Our results indicate that a considerable amount of enzyme activity can be recovered by UF, implying that the proposed approach may be a promising option in intensifying the current manufacturing procedure.

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Reference: Kovács, Z., Benjamins, E., Grau, K., Ur Rehman, A., Ebrahimi, M., & Czermak, P. (2013). Recent Developments in Manufacturing Oligosaccharides with Prebiotic Functions. In Biotechnology of Food and Feed Additives (pp. 257–295). Springer, Berlin, Heidelberg. DOI: https://doi.org/10.1007/10_2013_237