Potential of monolithic membrane pore-through-flow bioreactors for the production of GOS

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Motivation

- The use of prebiotics like galacto-oligosaccharides (GOS) in food industry has risen sharply due to the increased health awareness of consumers, GOS have a positive influence on the beneficial intestinal bacteria and therefore crucial for the bifidogenic effect [1]
- On industrial scale, GOS are produced from bovine lactose (included in the dairy industry side-product whey) with the enzyme β-galactosidase in batch reactors via transgalactosylation
- Costs of large-scale use of the free enzyme in batch processes are very high, limiting the application in industry, so the development of efficient continuous production strategies, e.g. by enzyme immobilization in membrane reactors are a promising option

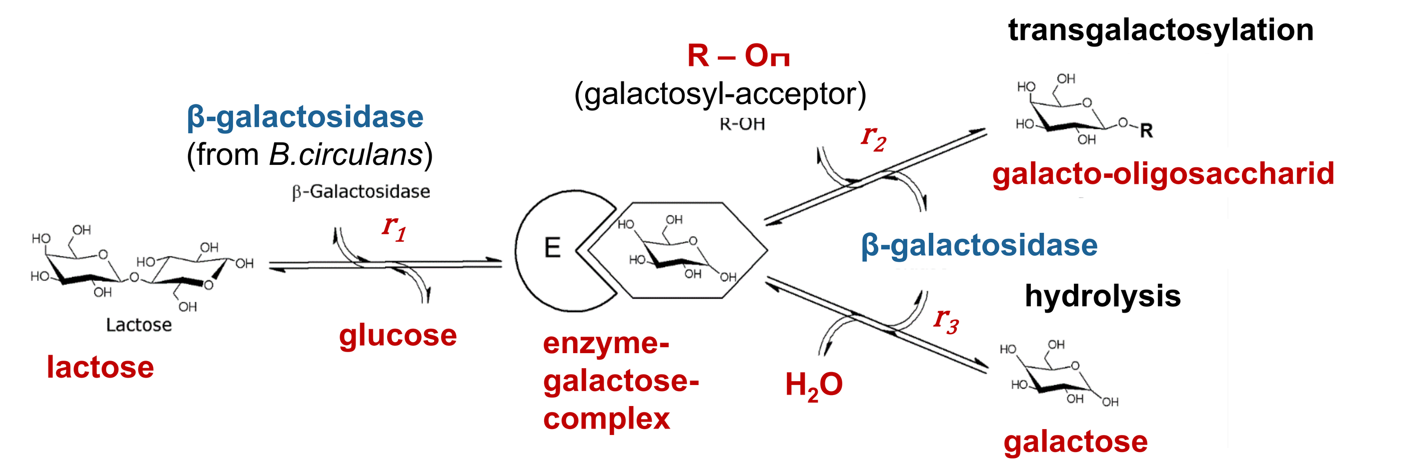
Enzyme - Immobilization

- Advantages: reusing the enzyme, opportunity to operate in continuous reaction systems, production of enzyme-free products
- Type of carrier is crucial for immobilization efficiency and mass transfer effects

Macroporous monoliths based on methacrylate are the most promising supports:

- Realizing a covalent enzyme binding, surface is activated with the chemical linker: carbonyldiimodazole (CDI), which generates the highest GOS-yield in screening experiments [2]
- Efficient mass transfer by convective pore flow without limitation by diffusion processes [3]
- Used as monolithic pore-through-flow reactors (PTFR) with different scales and operation modes

Equlibrium-limited reaction mechanism of GOS-synthesis



$$r_1 = -\frac{dc_{lac}}{dt} = f\,(c_{lac}, T, pH, E, Immob._{carrier})$$

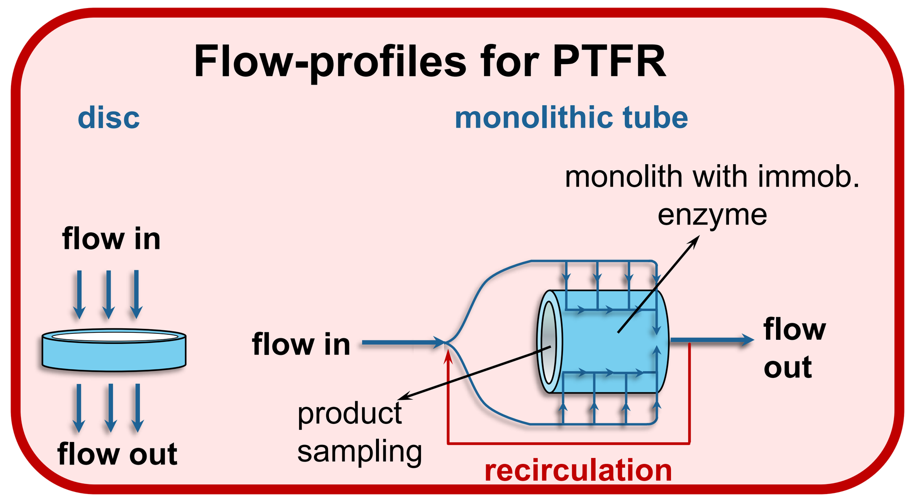
$$r_2 = \frac{dc_{GOS}}{dt} = f\,(c_{lac}, T, pH, E, Immob._{carrier})$$

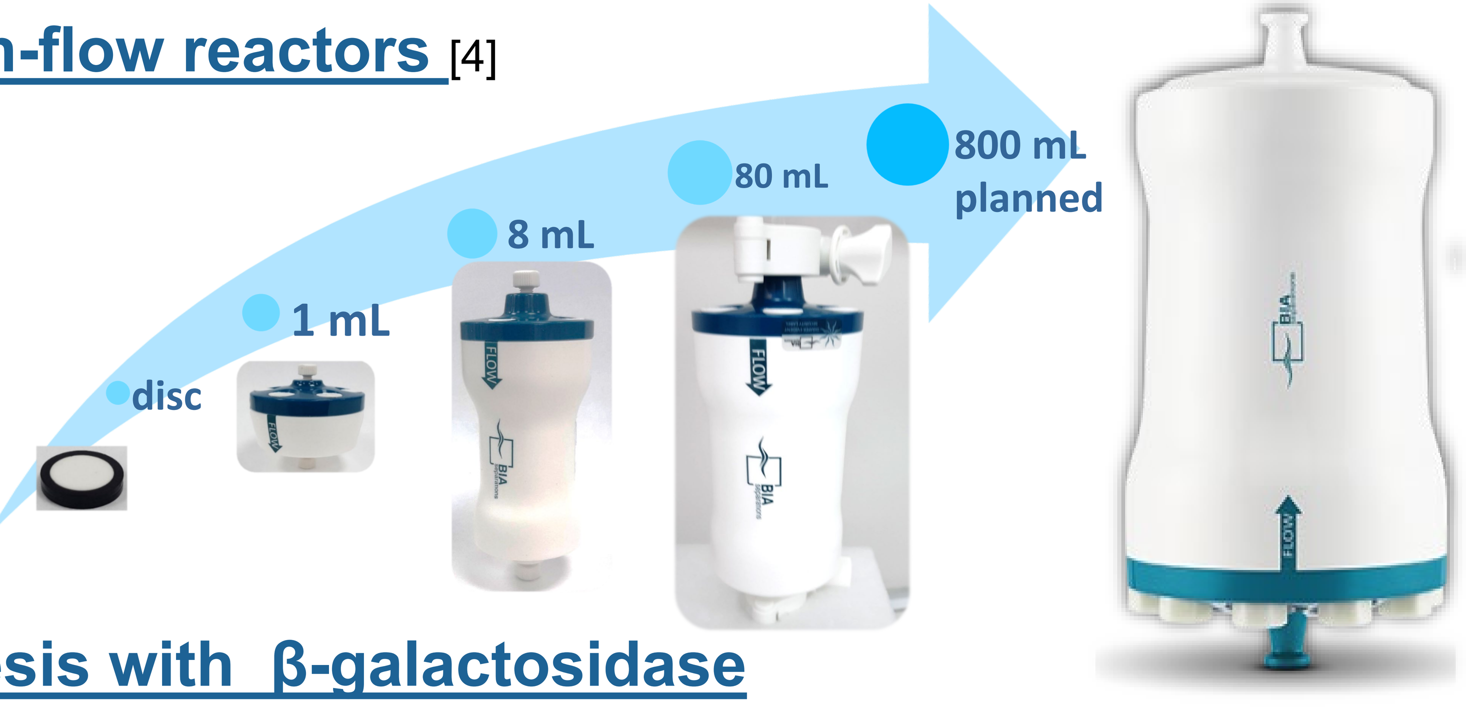
Parameter calculation for Michaelis-Menten kinetics:

- Provides basis for model based process optimization

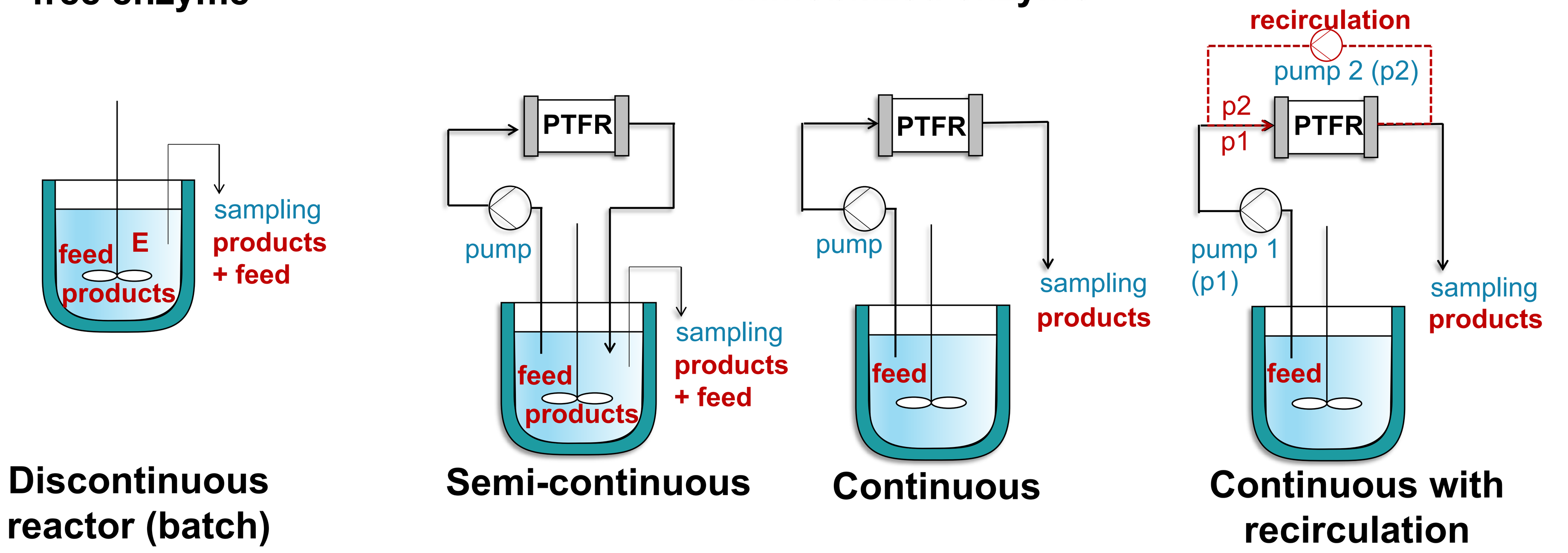
$$r_2 = \frac{dc_{GOS}}{dt} = \frac{v_{max,2} \cdot c_{GOS}}{K_{M,2} + c_{GOS}}$$

Scale-up monolithic pore-through-flow reactors [4]





Operation modes for GOS-synthesis with β-galactosidase



E: free enzyme

PTFR: pore-through-flow reactor with **immobilized enzyme**

feed: 100 g L^{-1} lactose (0.1 mol L^{-1} phosphate buffer+1 mM $MgCl_2$, pH 7.5)

products: **GOS** (with remaining lactose, glucose, galactose)

Characterisation and kinetic parameters of β-galactosidase

	free enzyme	Immobilzed enzyme on PTFR			
		disc	1 mL tube	8 mL tube	80 mL tube
real reactor volume*1 (mL)	40	0.1	0.3	2.4	24
enzyme (mg)	9.0	3.7	8.4	71	710
spec. activety*2 (U mg_{Enz}^{-1})	196	2	2	3	2
E/S (U g_{lac}^{-1})	49	750	567	850	594
$K_{M,2}$ (g L^{-1})	140	130	125	122	144
$V_{max,2}$ *3 (g (L min)$^{-1}$)	3	19	33	45	52

*1 PTFE:liquid pore volumes calculatet with porosity (ε = 0.3);*2 one Unit (U):conversion of 1 µmol lactose per min;*3 g GOS per L and min

free enzyme: Continuous stirred tank reactor

immobilized enzyme: Semi-continuous configuration; flow: 0.5 mL min^{-1} (disc), 1 mL min^{-1} (1 mL-tube), 10 mL min^{-1} (8 mL-tube)

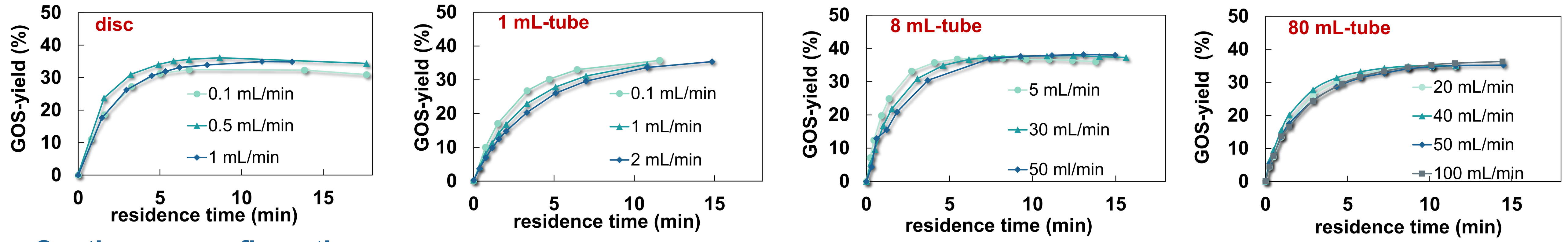
Calculation of residence time (contact between enzyme and substrate)

free enzyme: $\bar{t} = sampling\ time$

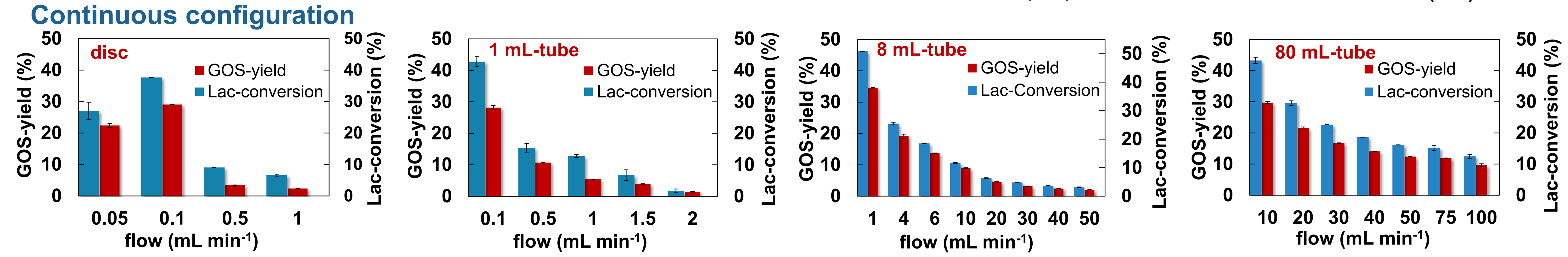
immobilized enzyme: $\bar{t} = \frac{V_{tot} \cdot \varepsilon}{\dot{V}} = \frac{L_{p,eff} \cdot \varepsilon}{w \cdot \tau}$

GOS-synthesis with immobilized β-Galactosidase

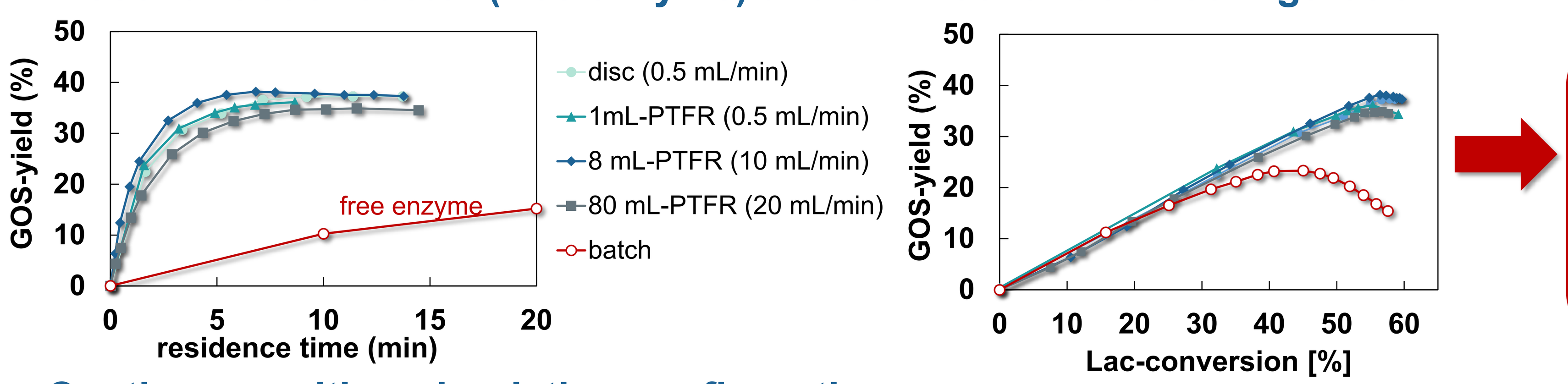
Semi-continuous configuration



Continuous configuration



Discontinuous reactor (free enzyme) and semi-continuous configuration PTFR (immob. Enzyme)



- Higher GOS-yield with immobilized enzyme: shift to transgalactosylation
- Scale-up from disc to 1 mL-, 8 mL- and 80 mL-tube successful
- Results directly transferable
- Further scale-up to 800 mL-PTFR is in progress

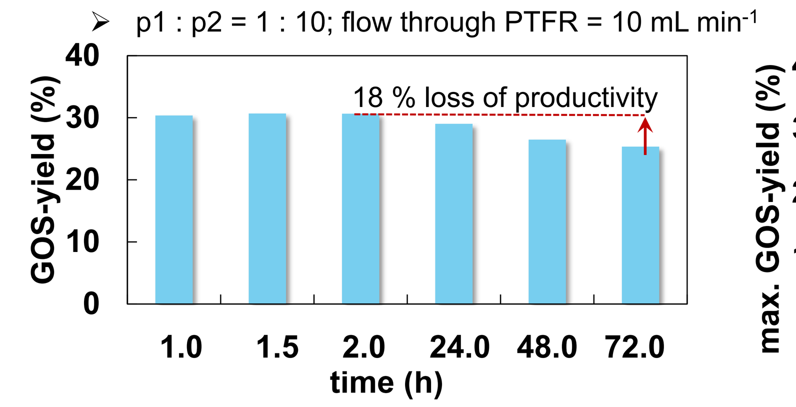
Continuous with recirculation configuration

Determination of ideal pump flow with 1 mL-tube

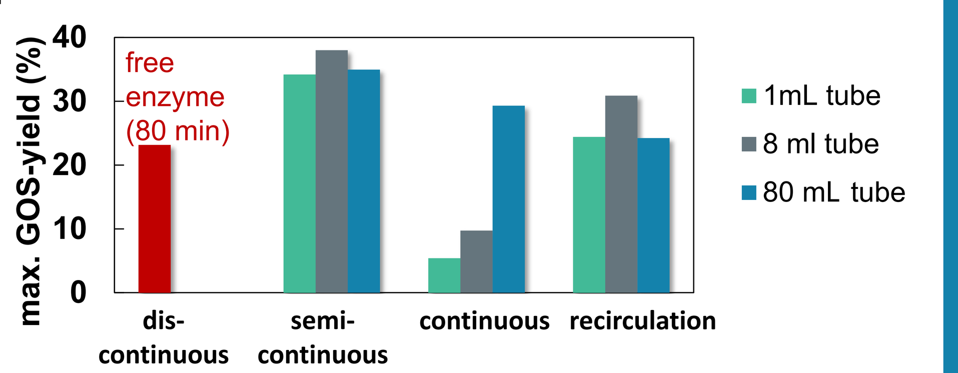
ratio p1 : p2	flow (mL min-1) p1	p2	through PTFR	max. GOS-yield (%) t = 120 min
1 : 10	0.09	0.91	1.00	20
1 : 10	0.06	0.6	0.66	25
1 : 5	0.17	0.83	1.00	15
1 : 5	0.10	0.50	0.60	21
1 : 2	0.33	0.67	1.00	10
1 : 2	0.10	0.20	0.30	22

transfer of results to 8 mL-tube

- p1 : p2 = 1 : 10; flow through PTFR = 10 mL min^{-1}



Comparison of different operation modes



- semi-cont.: 1 mL min^{-1} (1 mL-PTFR), 10 mL min^{-1} (8 mL-PTFR), 20 mL min^{-1} (80 mL-PTFR)
- contin.: 1 mL min^{-1} (1 mL-PTFR), 10 mL min^{-1} (8 mL-PTFR), 10 mL min^{-1} (80 mL-PTFR)
- recirculation: p1 : p2 = 1 : 10; flow through PTFR = 10 mL min^{-1}

- High recirculation rate (p2) + low feed-flow (p1) lead to highest GOS-yield
- with recirculation mode: higher GOS-yield can be achieved at same retention times like continuous mode, reduction of GOS-degradation as in semi-continuous mode obtained

Conclusion

- Monolithic membrane pore-through-flow bioreactors have significant potential for GOS-synthesis from lactose, furthermore parameter calculation as an outlook for additional simulation studies
- GOS-yield could be increased compared to application of free enzyme
- Semi-continuous and continuous configuration scale-up (from 1 mL- and 8 mL to 80 mL-tubes) proved to be successful: results are transferable directly
- Recycling system was systematically tested with different flow rates: optimal operating parameters with respect to highest GOS-yield obtained and transfered to 8 mL- and 80 mL-tubes

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References: [1] Tanaka, R., et al., Bifidobacteria and microflora, 1983. 2(1): p. 17-24; [2] Pottratz, I., et al., Chemie Ingenieur Technik, 2021, 93: p. 353-363; [3] Vodopivec, M., et al., Analytical Technologies in the Biomedical and Life Sciences, 2003. 795: p. 105-113; [4] BIASeparation: www.biaseparations.com