Supplementary Materials

Immobilization-Stabilization of β-Glucosidase for Implementation of Intensified Hydrolysis of Cellobiose in Continuous Flow Reactors

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Supplementary Materials Content

Supplementary Tables

Table S1. Technical specification of the material supports**.**

Support	Particle size, µm	Functional group	Other specifications
4% BCL Agarose Bead Standard (Ag 4BCL)	50-150	Plain crosslinked	Bead Mean Diameter d50v: ~90 µm
6% BCL Agarose Bead Standard (Ag 6BCL)	50-150	Plain cross-linked	Bead Mean Diameter d50v: ~90 µm
Lifetech™ ECR8209F (Pur)	150 - 300 µm	Epoxy	600 - 1200 Å pore diameter

*To be activated

Table S2. Fitting of the activity time course of β-glucosidase catalysts.

Biocatalyst	**Ag-PEI**	**Ag-Gly 4BCL**
Act_0 (%)	102.03 ± 0.02	100.00 ± 0.02
a_1	0.85 ± 0.01	0.69 ± 0.03
a_2	0.15 ± 0.01	0.31 ± 0.01
k_1 (h^{-1})	0.02 ± 0.01	0.02 ± 0.00
k_2 (h^{-1})	0.79 ± 1.37	2.16 ± 0.71
R^2	0.97	1

Table S3. Kinetic and statistical parameters of the different fittings of the kinetic models: Competitive product inhibition (Model 1), competitive substrate inhibition (Model 2) and competitive substrate inhibition and double competitive product inhibition (Model 3).

Model	Kinetic equation	k_{cat} (µmol/min/mg)	K_M (mM)	K_I (mM)	K_P (mM)	SQR	F
1	$r = \frac{k_{cat} C_{enz} C_{cel}}{C_{cel} + K_M \left(1 + \frac{2 \cdot (C_{cel0} - C_{cel})}{K_P}\right)}$	400	9.1	-	0.8	1353	2822
2	$r = \frac{k_{cat} C_{enz} C_{cel}}{C_{cel} + K_M \left(1 + \frac{2 \cdot (C_{cel0} - C_{cel})}{K_P}\right)}$	95.3	200	1000	-	19050	225
3	$r = \frac{k_{cat} C_{enz} C_{cel}}{K_M \cdot \left(1 + \frac{2 \cdot (C_{cel0} - C_{cel})}{K_P}\right)^2 + C_{cel} \cdot \left(1 + \frac{C_{cel}}{K_i}\right)}$	333.2	43.0	1088	34.0	2051	5400

Supplementary Figures

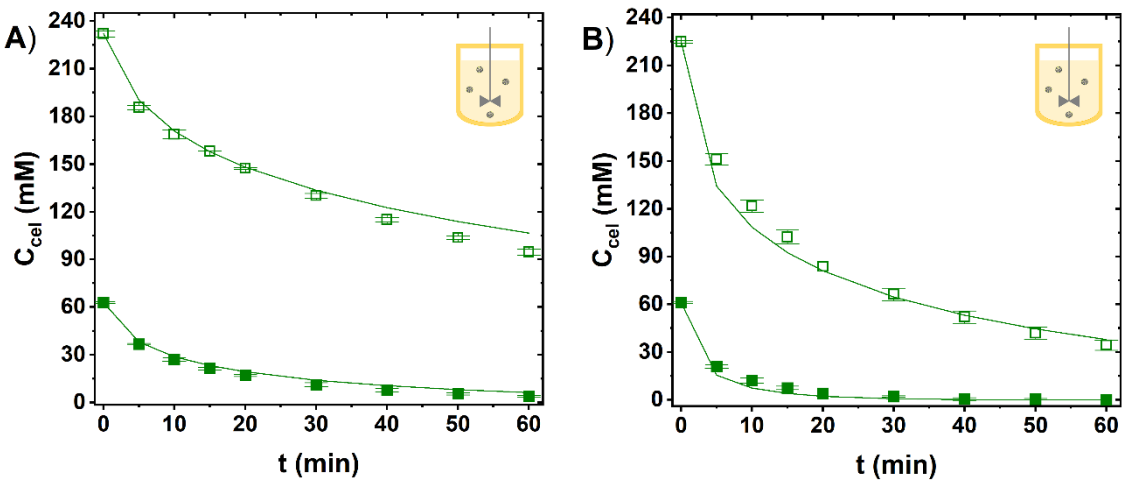

Figure S1. Concentration time course of the hydrolysis of cellobiose catalyzed by free enzyme at different enzyme concentrations. (**A**) 59.2 mg/L and (**B**) 296.1 mg/mL with different cellobiose concentrations (sodium citrate buffer 50 mM pH 5.0): 20 g/L (filled squares) and 70 g/L (empty squares). All experiments were performed in batch reactors at 50 ºC with a reaction volume of 20 mL. The solid line shows the fit of the kinetic model of substrate-competitive inhibition and product-competitive dual inhibition. Then, the integral method was applied (Figure 5) and different kinetic models of inhibition were fitted to experimental data, obtaining different kinetic parameters (Table S2). The best fitting model was a kinetic model featuring acompetitive substrate inhibition and double competitive product inhibition (proposed by M. Wojtusik [S1]) for the enzyme β-glucosidase, in particular for this specific enzyme cocktail (ASA-1000). This fit is represented by a solid line in Figure S2. The inhibition constant by product was low, so that there was hardly any substrate inhibition. A small product inhibition constant value was obtained, indicating that there was strong competitive inhibition by product.

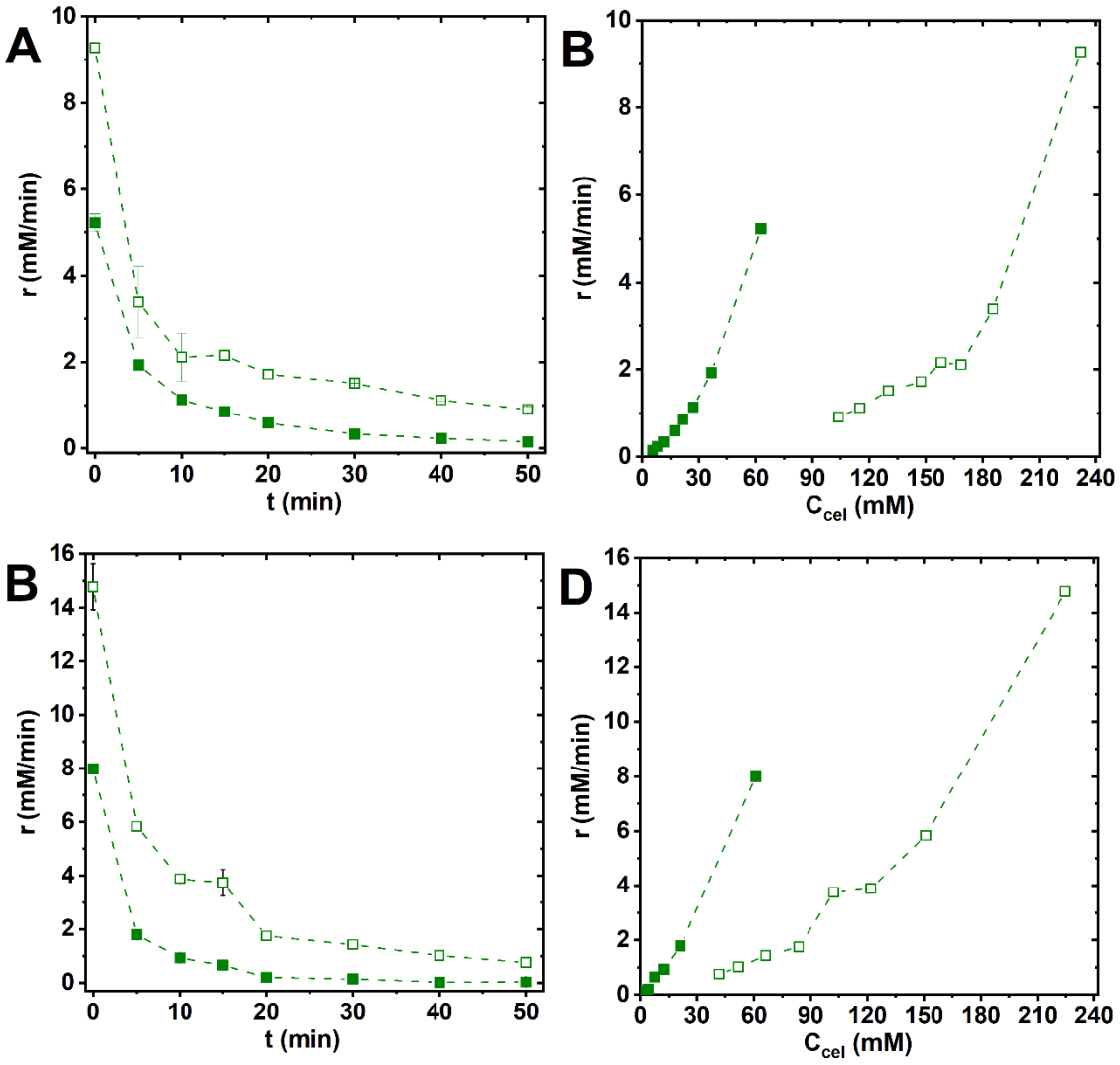

Figure S2. Reaction rate evolution of the hydrolysis of cellobiose catalyzed by free enzyme. Reaction rate vs time in the batch reactor (**A**,**C**). Reaction rate vs cellobiose concentration in the batch reactor (**B**,**D**). The enzyme concentration employed was 59.2 mg/mL (**A**,**B**) and 296.1 mg/mL (**C**,**D**). All data were obtained by the application of the differential method.

Supplementary References:

1. Wojtusik, M.; Yepes, C.M.; Villar, J.C.; Cordes, A.; Arroyo, M.; Garcia-Ochoa, F.; Ladero, M. Kinetic Modeling of Cellobiose by a β-Glucosidase from Aspergillus Fumigatus. *Chem. Eng. Res. Des.* **2018**, *136*, 502–512, doi:10.1016/j.cherd.2018.06.020.