

Nucleation studies of *Lactobacillus brevis* alcohol dehydrogenases in a stirred crystallizer monitored by in situ multi-angle dynamic light scattering (MADLS)

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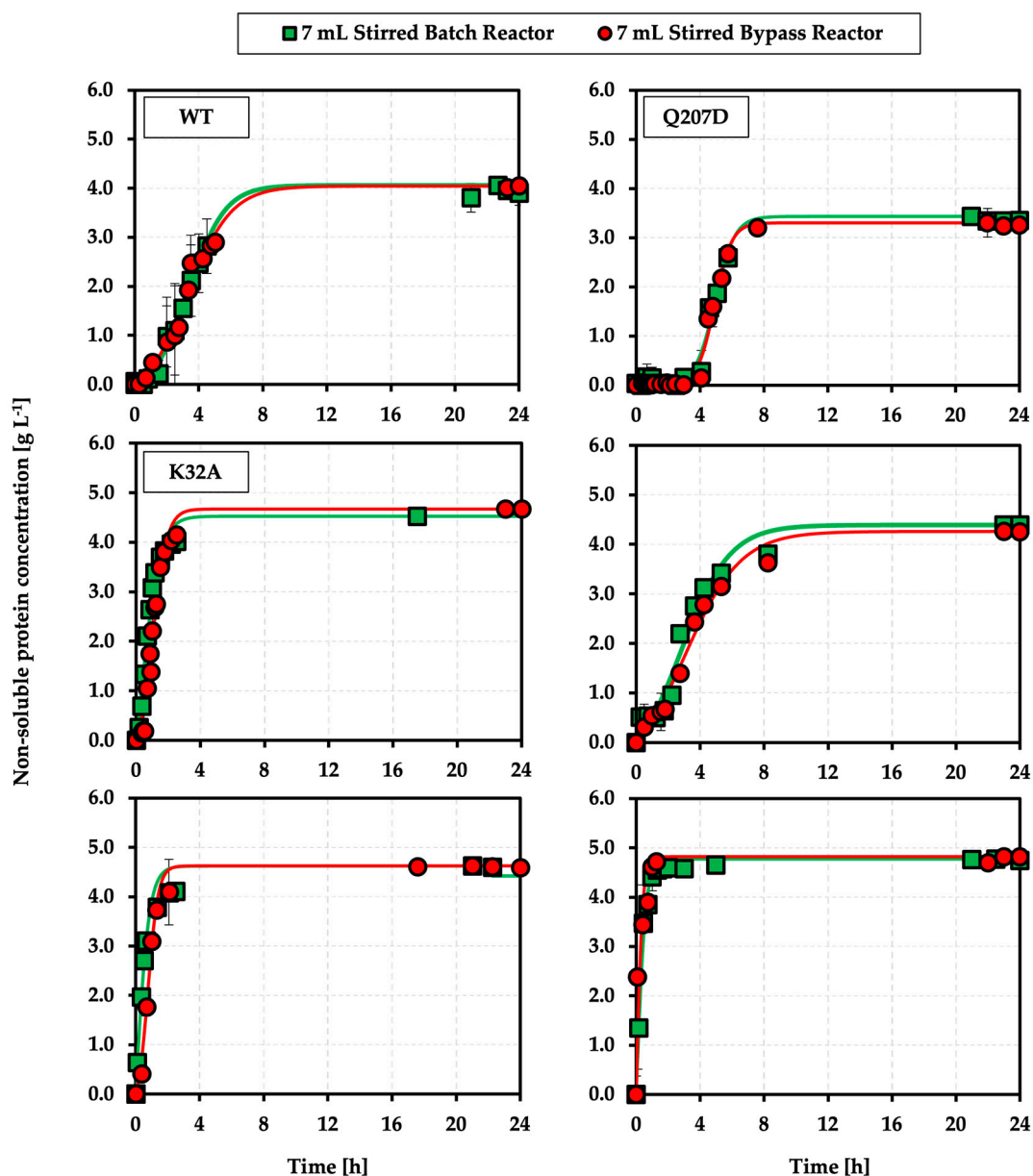


Figure S1. Comparison of stirred 7 mL batch and 7 mL flow-through crystallizers for *LbADH* WT and crystal-contact variants. Time evolution of the non-soluble protein concentration during crystallization of (a) WT, (b) Q207D, (c) K32A, (d) Q126H, (e) D54F and (f) T102E. Green symbols denote experiments performed in the standard stirred 7 mL batch crystallizer, red symbols denote experiments in the stirred 7 mL crystallizer integrated into the external MADLS flow-through setup. Solid lines represent logistic fits according to Equation (3). ($c_0 = 5 \text{ g L}^{-1}$, 100 g L^{-1} PEG 550 MME, $n_s = 150 \text{ rpm}$, 50 mM Tris-HCl, 25 mM MgCl₂, 10 mM HEPES-NaOH, pH 7.0, $T = 20 \text{ }^\circ\text{C}$).

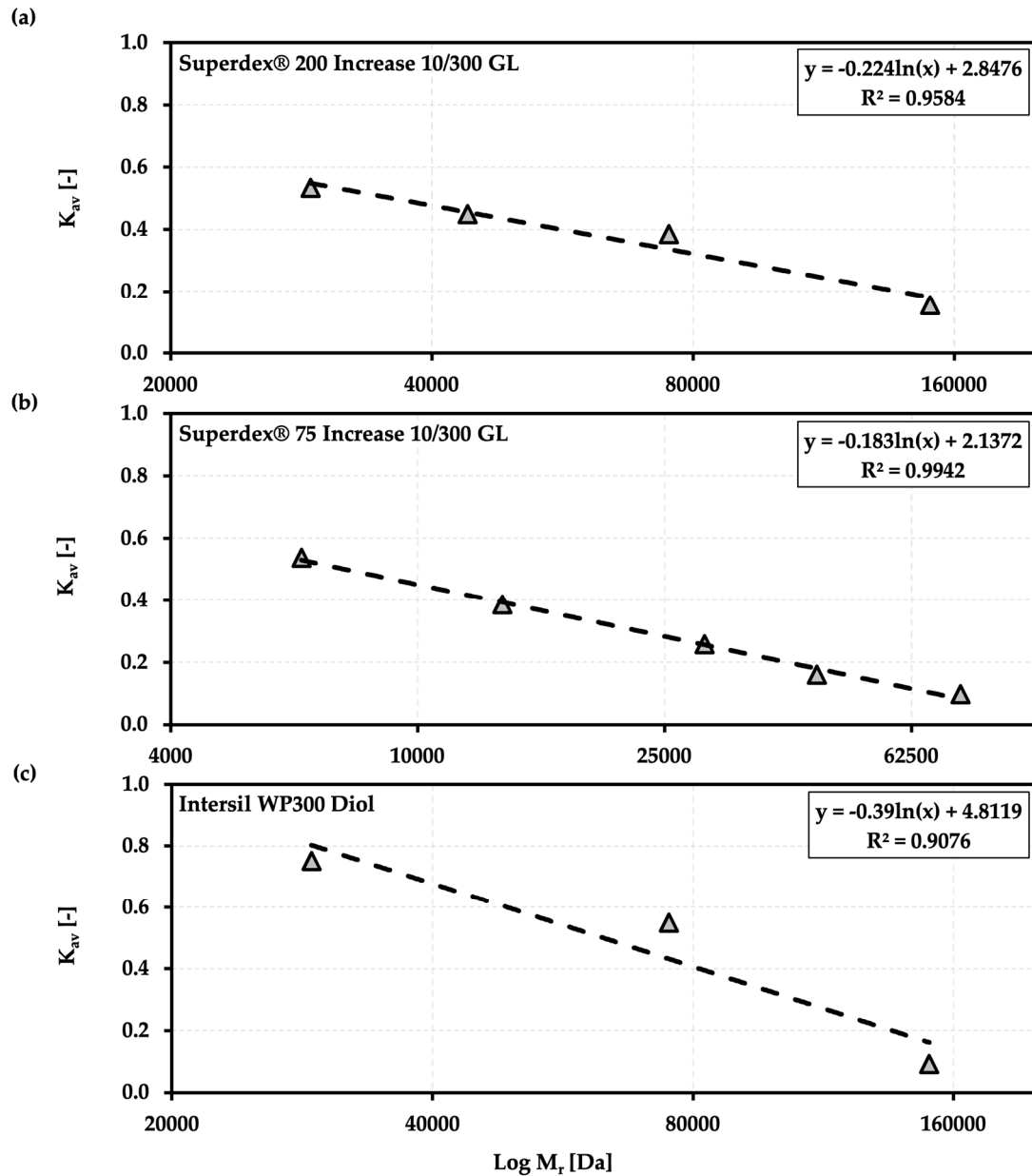


Figure S2. Calibration of the chromatographic columns used for SEC and SE-HPLC analysis of the *Lb*ADH. **(a)** Calibration curve for the size-exclusion column with a separation range of approximately 10 - 200 kDa for globular proteins (Superdex® 200 Increase 10/300 GL), obtained from injections of the low-molecular-weight gel filtration standard. **(b)** Corresponding calibration for the column with a separation range of approximately 3 - 75 kDa (Superdex® 75 Increase 10/300 GL). **(c)** Calibration of the SE-HPLC column with an effective separation range up to about 650 kDa (Inertsil WP300 Diol) using a protein standard mixture. Symbols denote experimental data points and solid lines the linear regression fits. The respective fit equations and coefficients of determination are indicated in each panel and were used to convert elution positions into apparent molecular masses in the main text.

Table S1. Comparison of the crystallization variables and results with the starting concentration c_0 , the equilibrium concentration c_{eq} after 24 h, and the yields Y of the *Lb*ADH WT and crystal-contact variants obtained from 7 mL stirred batch reactor and 7 mL stirred bypass reactor crystallizations. The values are calculated from the mass balance using Equation 2. The parameters of the logistic fit functions of Equation 3 are also shown (maximum concentration $c_{max,X}$, crystal growth factors k_X , and inflection points $t_{1/2,X}$). Furthermore, the coefficient of determination R^2 and the calculated root mean square deviations (RMSD) between the fitted functions are given to illustrate the deviations. ($c_0 = 5 \text{ g L}^{-1}$, 100 g L^{-1} PEG 550 MME, $n_s = 150 \text{ rpm}$, 50 mM Tris-HCl, 25 mM MgCl_2 , 10 mM HEPES-NaOH, $\text{pH } 7.0$, $T = 20 \text{ }^\circ\text{C}$).

<i>Lactobacillus brevis</i> ADH		Q207D	WT	Q126H	K32A	D54F	T102E
Batch Reactor	Crystallization Parameters	c_0 [g L ⁻¹]	5.02 ± 0.02	5.11 ± 0.14	5.00 ± 0.00	4.96 ± 0.00	5.00 ± 0.00
		c_{eq} [g L ⁻¹]	1.66 ± 0.04	1.24 ± 0.11	0.61 ± 0.00	0.43 ± 0.00	0.39 ± 0.01
		Y [%]	66.80	75.82	87.80	91.32	92.20
	Logistic parameters $c_X(t)$	$c_{max,X}$ [g L ⁻¹]	3.43	4.059	4.39	4.53	4.62
		k_X [h ⁻¹]	1.64	0.89	0.66	1.76	2.02
		$t_{1/2,X}$ [h]	4.94	3.40	2.82	0.34	0.01
		R^2 [-]	0.99	0.99	0.97	0.98	0.98
	Crystallization Parameters	c_0 [g L ⁻¹]	5.03 ± 0.2	5.12 ± 0.21	5.00 ± 0.31	5.11 ± 0.29	5.00 ± 0.19
		c_{eq} [g L ⁻¹]	1.76 ± 0.3	1.10 ± 0.02	0.75 ± 0.01	0.44 ± 0.00	0.39 ± 0.02
		Y [%]	64.94	78.63	85.10	91.39	92.19
Bypass Reactor	Crystallization Parameters	c_0 [g L ⁻¹]	5.03 ± 0.2	5.12 ± 0.21	5.00 ± 0.31	5.11 ± 0.29	5.00 ± 0.19
		c_{eq} [g L ⁻¹]	1.76 ± 0.3	1.10 ± 0.02	0.75 ± 0.01	0.44 ± 0.00	0.39 ± 0.02
		Y [%]	64.94	78.63	85.10	91.39	92.19
	Logistic parameters $c_X(t)$	$c_{max,X}$ [g L ⁻¹]	3.30	4.04	4.26	4.67	4.63
		k_X [h ⁻¹]	1.89	0.74	0.57	2.26	3.16
		$t_{1/2,V}$ [h]	4.92	3.32	3.06	1.04	0.78
		R^2 [-]	0.99	0.99	0.99	0.99	0.99
		$RMSD$ [-]	0.011	0.016	0.029	0.026	0.054
	Crystallization Parameters	c_0 [g L ⁻¹]	5.03 ± 0.2	5.12 ± 0.21	5.00 ± 0.31	5.11 ± 0.29	5.00 ± 0.19
		c_{eq} [g L ⁻¹]	1.76 ± 0.3	1.10 ± 0.02	0.75 ± 0.01	0.44 ± 0.00	0.39 ± 0.02
		Y [%]	64.94	78.63	85.10	91.39	92.19
	Logistic parameters $c_X(t)$	$c_{max,X}$ [g L ⁻¹]	3.30	4.04	4.26	4.67	4.63
		k_X [h ⁻¹]	1.89	0.74	0.57	2.26	3.16
		$t_{1/2,V}$ [h]	4.92	3.32	3.06	1.04	0.78
		R^2 [-]	0.99	0.99	0.99	0.99	0.99
		$RMSD$ [-]	0.011	0.016	0.029	0.026	0.054