Supplementary material

Redox engineering by ectopic overexpression of NADH kinase in recombinant *Pichia pastoris (Komagataella phaffii)*: Impact on cell physiology and recombinant production of secreted proteins

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Figure S1. Representation of NADPH pool turnover in relation to Fab productivity. A series of FSOF simulations were performed by increasing the Fab specific productivity. A sum-flux analysis of NADPH was performed for each simulation and resulting NADPH turnovers are plotted into the graph.

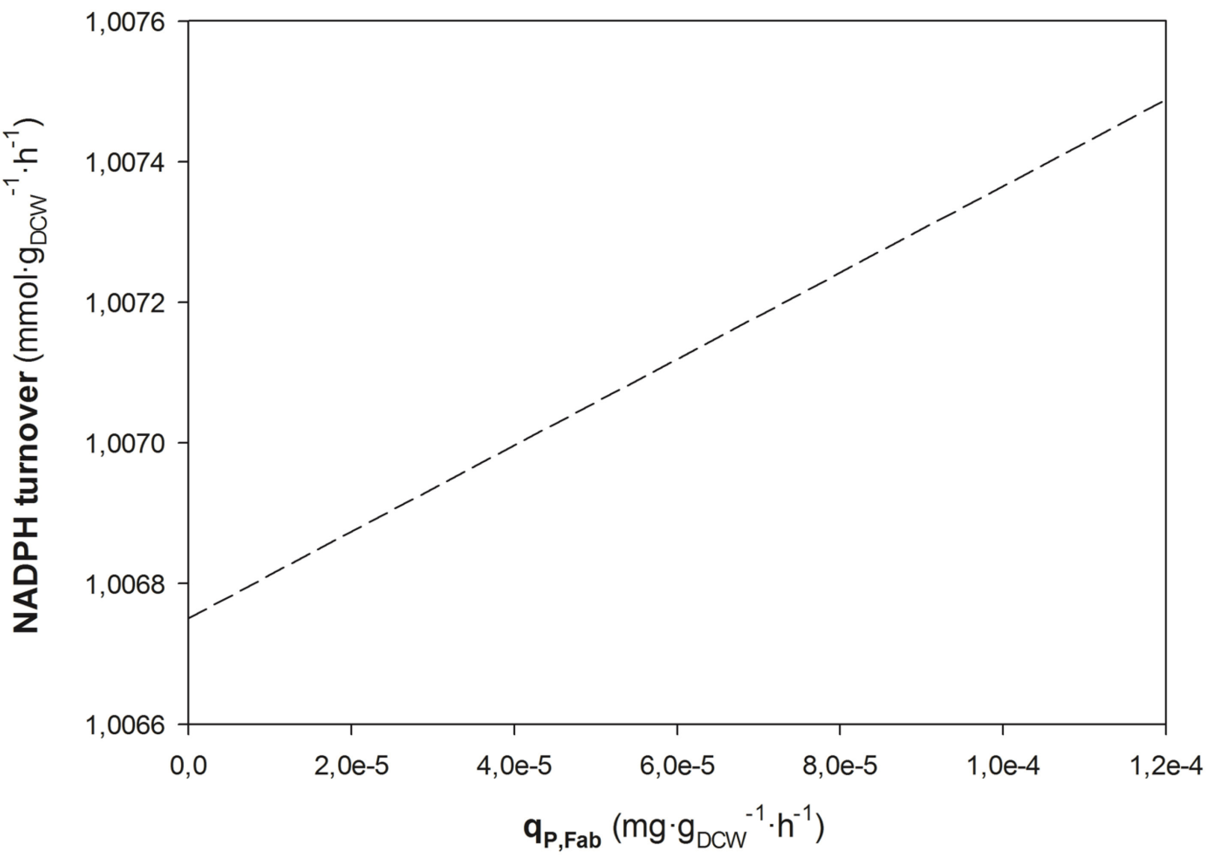

Table S1. Macroscopic growth parameters of cultivations performed in glycerol and normoxic conditions and glucose in normoxia and hypoxia after the reconciliation procedure. Values are average ± standard deviation of two independent replicates.

	Glycerol - normoxia				Glucose - normoxia				Glucose - hypoxia			
	X-33/2F5	cPOS5	2cPOS5	mPOS5	X-33/2F5	cPOS5	2cPOS5	mPOS5	X-33/2F5	cPOS5	2cPOS5	mPOS5
Glucose [a]	-	-	-	-	-0.80 ± 0.02	-0.93 ± 0.06	-0.93 ± 0.04	-0.93 ± 0.18	-1.20 ± 0.01	-1.26 ± 0.13	-1.37 ± 0.01	-1.03 ± 0.12
Glicerol [a]	-1.49 ± 0.06	-1.52 ± 0.16	-1.53 ± 0.01	-1.51 ± 0.23	0.01 ± 0.01	-	-	-	-	0.01 ± 0.01	-	-
Arabitol [a]	-	-	-	-	-	-	-	-	0.04 ± 0.01	0.07 ± 0.01	0.06 ± 0.01	0.04 ± 0.02
Ethanol [a]	-	-	-	-	-	-	-	-	0.53 ± 0.01	0.46 ± 0.11	0.70 ± 0.14	0.24 ± 0.17
Succinic [a]	-	-	-	-	-	-	-	-	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	-
Biomass [a]	3.36 ± 0.01	3.42 ± 0.20	3.41 ± 0.08	3.28 ± 0.34	3.15 ± 0.47	3.75 ± 0.03	3.80 ± 0.04	3.64 ± 0.37	3.54 ± 0.04	3.67 ± 0.15	3.76 ± 0.04	3.57 ± 0.21
CO2 [a]	1.10 ± 0.18	1.15 ± 0.26	1.19 ± 0.13	1.23 ± 0.36	1.60 ± 0.27	1.75 ± 0.24	1.81 ± 0.21	1.68 ± 0.35	2.00 ± 0.33	2.23 ± 0.23	2.36 ± 0.72	1.77 ± 0.24
O2 [a]	1.85 ± 0.21	1.91 ± 0.35	1.95 ± 0.15	1.98 ± 0.47	1.52 ± 0.28	1.67 ± 0.23	1.72 ± 0.21	1.59 ± 0.33	1.36 ± 0.26	1.63 ± 0.15	1.51 ± 0.33	1.37 ± 0.26
RQ [b]	0.60 ± 0.03	0.60 ± 0.03	0.61 ± 0.02	0.62 ± 0.03	1.05 ± 0.01	1.05 ± 0.01	1.05 ± 0.01	1.06 ± 0.01	1.48 ± 0.05	1.37 ± 0.02	1.55 ± 0.13	1.30 ± 0.07
Y_{xs} [c]	0.70 ± 0.03	0.70 ± 0.03	0.69 ± 0.02	0.68 ± 0.04	0.59 ± 0.07	0.62 ± 0.02	0.60 ± 0.04	0.62 ± 0.02	0.44 ± 0.01	0.44 ± 0.07	0.41 ± 0.01	0.53 ± 0.09
Fab [d]	0.02 ± 0.01	0.04 ± 0.02	0.04 ± 0.01	0.04 ± 0.03	0.02 ± 0.01	0.04 ± 0.03	0.05 ± 0.02	0.05 ± 0.03	0.07 ± 0.03	0.07 ± 0.04	0.12 ± 0.06	0.05 ± 0.02

[a] mmol$\cdot g_{DCW}^{-1}\cdot h^{-1}$
[b] mmol $CO_2\cdot g_{DCW}^{-1}\cdot h^{-1}$ / mmol $O_2\cdot g_{DCW}^{-1}\cdot h^{-1}$
[c] g biomass / g substrate
[d] mg Fab$\cdot g_{DCW}^{-1}\cdot h^{-1}$