Towards a novel computer-aided optimization of microreactors: Techno-economic evaluation of an immobilized enzyme system

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Supporting Information

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Abbreviations

Notation

Btn	Biotin	$\widetilde{M}_i$	Molecular weight of component i, $g\ mol^{-1}$
c_i	Concentration of component $i, mol\ L^{-1}$	MB	Magnetic bead
CapEx	Capital expenditure €	NADP	Nicotinamid-adenin-dinukleotid-phosphat
CFD	Computational fluid dynamics	NDK	5-nitrononan-2,8-dion
CH	Chlorohexane	N_i	Substance amount of component i, mol
Diol	5-nitronona-2,8-diol	OpEx	Operating expenditure $€h^{-1}$
D_r	Mechanism specific denominator	OT	Operation time h
D_r^{reg}	Regulation term	PEG	Polyethylenglycol
d_{bed}	Thickness of the bed	$price_i$	Price of component $i, € g^{-1}$
d_h	Hydraulic diameter	$Prod_{cat}$	Biocatalytic productivity
d_P	Diameter of the magnetic particles	ST	Spy-tag
F2D	2D Fluent model	STV	Streptavidin
F3D	3D Fluent model	STY	Space time yield, $kg\ L^{-1}s^{-1}$
GDH	Glucose dehydrogenase	t	Time, s
Gluacid	Gluconolacton	T_r	numerator, s^{-1}
Gre2	Ketoreductase	TEP	Techno-Economic Performance
HK	8-hydroxy-5-nitrononan-2-on	u	Flow velocity, $m\ s^{-1}$
HOB	Halo-based oligonucleotide binder	V_{bed}	Volume of the packed-bed, m^3
His	Histadin	V_{ec}	Volume of the empty channel, m^3
$k_{cat}^{\pm}$	Turnover rate (+ for forward, - for backward reactions), s^{-1}	V_i	molecular volume, cm^3
K_{eq}	Equilibrium constant	$\dot{V}$	Flow rate $m^3 s^{-1}$
K_M^i	Affinity constant (Michaelis-Menten constant) of component i, $mol\ L^{-1}$	W	Channel width, m
K_A^i	Activation constant of component i, s^{-1}	Y	Yield
K_I^i	Inhibition constant of component i, s^{-1}	y	Position perpendicular to the flow direction, m
L	Reactor length		

Greek letters

η	Enzyme utilization (EU)
τ	space time, s
v_r	reaction rate, $mol\ L^{-1}s^{-1}$
v_0	initial reaction rate, $mol\ L^{-1}s^{-1}$

Materials and Methods

S1 Enzyme immobilization

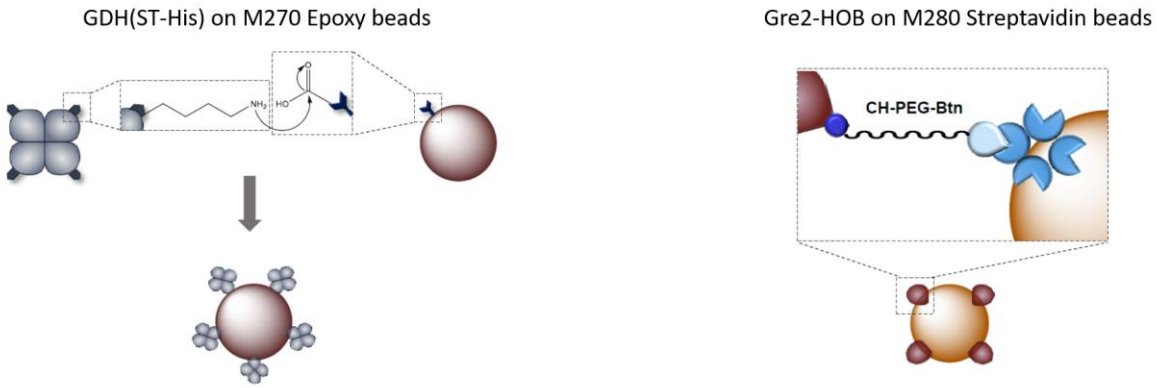

Figure S1 Immobilization methods for GDH(ST-His) (left) on Epoxy MBs and Gre2-HOB (right) on Streptavidin MBs.

The immobilization strategies for GDH and Gre2 are visualized by Figure S1. Streptavidin (STV)-coated MBs (Dynabeads™ M-280 Streptavidin, Invitrogen) were used for Gre2-HOB immobilization according to manufacturer's instructions. The procedure was described previously [1]. In brief, MBs were functionalized with chlorohexane (CH) ligand by incubation with the Biotin (Btn)-PEG-CH conjugate in T-TEMg buffer.

GDH was immobilized covalently utilizing a Spy-Tag (ST) and hexahistidin-Tag (His) on Epoxy MBs (Dynabeads™ M-270 Epoxy). For both immobilization methods, beads were collected with a magnet, the supernatant was removed, and the beads were washed using the buffer solution.

For both Gre2 and GDH, functionalized MBs were mixed with 1.6 nmol Gre2-HOB per mg beads and 100 pmol GDH-His per mg beads, respectively. The mixtures were incubated on a rotary shaker at 30 °C for at least 30 min. Subsequently, beads were collected using a magnet, the supernatant was removed, and the beads were washed three times with T-TEMg.

S2 Determination of MB loading capacity

The capacity of Gre2 and GDH on magnetic beads were determined separately, due to different immobilization methods. Determinations were performed according to literature [2].

The amount of Gre2-HOB on the MBs was derived for non-covalent STV-Btn. Bonds between CH-PEG-Btn and STV-coated MBs were cleaved by heating the enzyme-functionalized MBs in SDS to 95 °C. The samples as well as calibration samples with defined amounts of corresponding purified protein were analyzed by standard SDS-polyacrylamide gel electrophoresis. Comparative greyscale

analysis was performed using ImageJ 1.48v software. Enzyme loading for Gre2 on streptavidin beads was previously determined to be 24 pmol/g [1].

For covalently bound GDH(ST-His), proteins on the MBs were determined with the remaining supernatant after the immobilization process. The supernatant with unbound GDH (ST-His) is separated and analyzed by standard SDS-polyacrylamide gel electrophoresis in parallel to calibration samples. Comparative greyscale analysis resulted in the amount of unbound proteins. The difference between feed and the remaining amount of GDH results in the GDH enzyme loading of the MBs which was determined as 55 pmol/mg.

S3 Fluent model

The basic Matlab model was transferred to ANSYS Fluent to constitute model F2D. A homogeneous, porous bed was assumed. To address effective diffusion within the catalytic bed, porosity and tortuosity were calculated for an ordered cubic particle packing according to the basic model [1]. Kinetic parameters and models were implemented with a User-Defined-Function for the stiff-chemistry solver. For the reactor, a hexahedral mesh was created. In order to reduce discretization errors, regional refinements in regions with high concentration gradients were performed. These areas are located at the top of the catalytic layer, as indicated by calculations with the basic matlab model. Additionally, near wall and transition regions from the bed to the free channel volume were selected for refinement, based on the flow velocity gradient within the free volume of the reactor. All walls and the top of the catalytic layer were assigned with no-slip boundary conditions. In- and outlet of the bed volume were assumed to be impermeable walls, since convective transport in the packed bed is neglectable, due to the flow resistance within the bed, resulting by the size of the particles. Liquid velocity was fixed at the inlet with a previously calculated, fully developed laminar flow. The pressure at the outlet was set to atmospheric.

Stationary velocity fields in the free volume were calculated separately and fixed for the species transport calculations, to reduce computational effort. In order to derive a stationary solution for species transport and reaction, transient calculations were performed until outlet concentrations achieved stationarity.

The same procedure was applied for model F3D. Since an increase in dimension has a high impact on the amount of cells, the length of the calculated region was reduced. Walls at the sides were assigned no-slip boundary conditions. A symmetry plane in the middle of the reactor was used to reduce the amount of cells of the model.

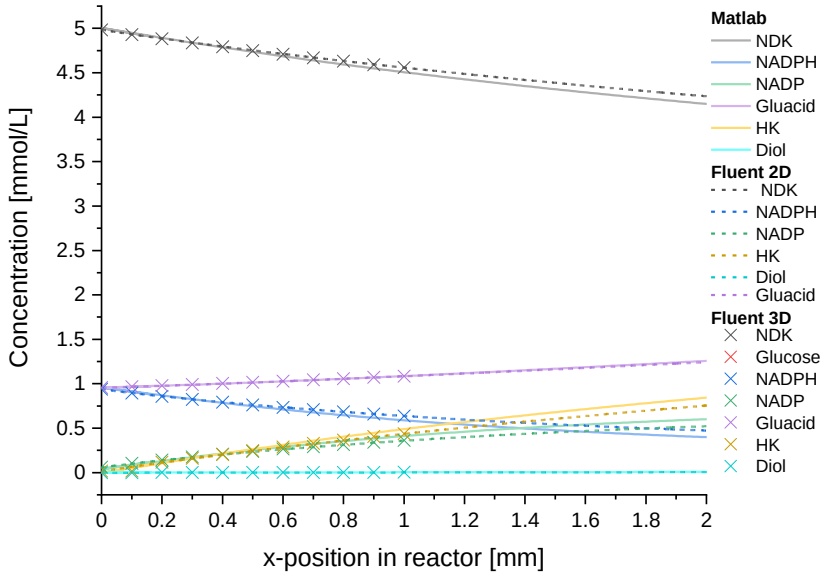

Figure S2 Development of the cross section averaged concentration in the free channel volume in flow direction for the basic Matlab model (lines), model F2D (dashes) and model F3D (crosses).

S4 Reaction kinetics

Kinetic parameters derived by Burgahn et. al. were applied in this work [1]. The governing reactions were implemented as with multisubstrate kinetics according to Equation (1), and a mechanistically based expression by Liebermeister et. al. was applied [3].

$$A + B \overset{E}{\leftrightarrow} P + Q \tag{1}$$

The general reaction rate v_r depends on the enzyme concentration c_{Enzyme}, the numerator T_r and the denominator $D_r^{reg} + D_r$ are chose (eqs. 2 and 3).

$$v_r = c_{Enzyme} \cdot \frac{T_r}{\left(D_r^{reg} + D_r\right)} \tag{2}$$

With:

$$T_r = k_{cat}^{+} \frac{c_A}{K_M^A} \frac{c_B}{K_M^B} - k_{cat}^{-} \frac{c_P}{K_M^P} \frac{c_Q}{K_M^Q} \tag{3}$$

Depending on the mechanism, the denominator D_r is adjusted with the respective Equations (4)-(6). Haldane relationships and Wegscheider conditions are applied to take thermodynamics in

consideration [4]. As mentioned by Burgahn et al., for reduction an irreversible ordered mechanism and for cofactor regeneration a reversible random-ordered mechanism was applied with the reaction parameters from table 1.

$$D_r^{reg} = \sum_i \left(\frac{K_A^i}{c_i}\right) + \sum_i \left(\frac{K_I^i}{c_i}\right) \tag{4}$$

$$D_{r,rand} = \left(1 + \frac{c_A}{K_M^A}\right)\left(1 + \frac{c_B}{K_M^B}\right) + \left(1 + \frac{c_P}{K_M^P}\right)\left(1 + \frac{c_Q}{K_M^Q}\right) - 1 \tag{5}$$

$$D_{r,ord} = 1 + \frac{c_A}{K_M^A} + \frac{c_Q}{K_M^Q} + \frac{c_A}{K_M^A}\frac{c_Q}{K_M^Q} + \frac{c_P}{K_M^P} + \frac{c_B}{K_M^B} + \frac{c_P}{K_M^P}\frac{c_B}{K_M^B} + \frac{c_A}{K_M^A}\frac{c_P}{K_M^P} + \frac{c_B}{K_M^B}\frac{c_Q}{K_M^Q} + \frac{c_A}{K_M^A}\frac{c_P}{K_M^P}\frac{c_B}{K_M^B} + \frac{c_B}{K_M^B}\frac{c_P}{K_M^P}\frac{c_Q}{K_M^Q} \tag{6}$$

Table S1 Kinetic parameters for NDK reduction and cofactor regeneration. Given are association constants (K_m^i) and rate constants ($k_{cat}^{\pm}$) for the reaction network. Values were derived by Burgahn et. al. [1]. In case of Gre2, materials NDK, HK and Diol may compete for the binding pocket. Therefore, inhibition constants were calculated for the respective reduction reactions by fitting the kinetic parameters to the experimental data. These simulated values are indicated by an asterisk (*).

	K_M^A [mM]	K_M^B [mM]	K_M^P [mM]	K_M^Q [mM]	k_{cat}^+ [1/s]	k_{cat}^- [1/s]
Cofactor Regeneration (GDH, sol./imm)[a]	40.19	0.76	0.97	1.01	5.77	0.92
1. Reduction from NDK to HK (Gre2,/imm)[b]	5.07	$7.14 \cdot 10^{-3}$	$5.72 \cdot 10^{-2}$ *	19.19*	9.09[d]	-
2. Reduction from HK to Diol (Gre2, imm.)[c]	66.58	$1.93 \cdot 10^{-2}$	$9.52 \cdot 10^{-4}$ *	1.92*	1.04[d]	-

a) A: glucose; B: NADP; P: gluconolactone; Q: NADPH
b) A: NDK; B: NADPH; P: HK; Q: NADP
c) A: HK; B: NADPH; P: Diol; Q: NADP
d) The velocity of the reaction step (k_{cat}) was assumed identical for immobilized and dissolved Gre2.

S5 Cost analysis

Contributing factors to the cost analysis are listed in Table S2 (CapEx) and Table S3 (OpEx). The costs for immobilized enzymes were calculated according to Tufvesson et al. with a factor of 1.9 as the immobilization increases the production costs (Tufvesson et al. 2011). The prices for the enzymes were chosen according to information provided by the vendors. This marks an upper limit for the enzyme costs, since producing a biocatalyst is usually cheaper than acquiring it from a vendor. However, enzyme costs represent 11% of the total CapEx, due to the small amount of immobilized

enzymes and the high bead costs. The major contributions is for Gre2 since this enzyme and the corresponding beads are more expensive than for GDH.

Table S2 Material prices for CapEx estimations in €/g. References are indicated and listed below the table.

Material	Price [€/g]
Dynabeads M280 (Gre2-MB)	19,000[a]
Dynabeads M270 (GDH-MB)	4,760[a]
Gre2 (imm.)	1,832,000[b]
GDH (imm.)	1,900[c]

a: Recieved from Thermo-Fischer [5]
b: Unmodified Gre2 according to MyBioSource [6], and additional costs for immobilization calculated according to Tufvesson et. al. [7]
c: Unmodified Gre2 according to Innosyn [8], and additional costs for immobilization calculated according to Tufvesson et. al. [7]

Table S3 Cost of chemical species for OpEx estimation in €/g. References are indicated and listed below the table.

Material	Price [€/g]
Glucose	0.05[a]
GDH(aq.)	1,000[b]
NADP	434[a]
NADPH	1,330[a]

a: Recieved from Sigma-Aldrich [9]
b : Recieved from Innosyn [8]

S6 Level 1 - calculations

A genetic algorithm was applied to the basic Matlab model [10]. Deriving optimal NADP feed concentrations for the infinitive fluxes case 1.3 was investigated. The resulting reactor behaviors for a fixed bead ratio and different NADP feed concentrations are depicted in Figure S3 A. A clear maximum at a concentration of 9 mmol/L is observed. Due to infinite regeneration rates, different bead ratios don't affect the reactor behavior, hence this was fixed at 0.74. Case 1.2 conditions were used to derive an optimal bead ratio. As shown in Figure S3 B, an optimum is found for the NADP/H concentration and bead ratio.

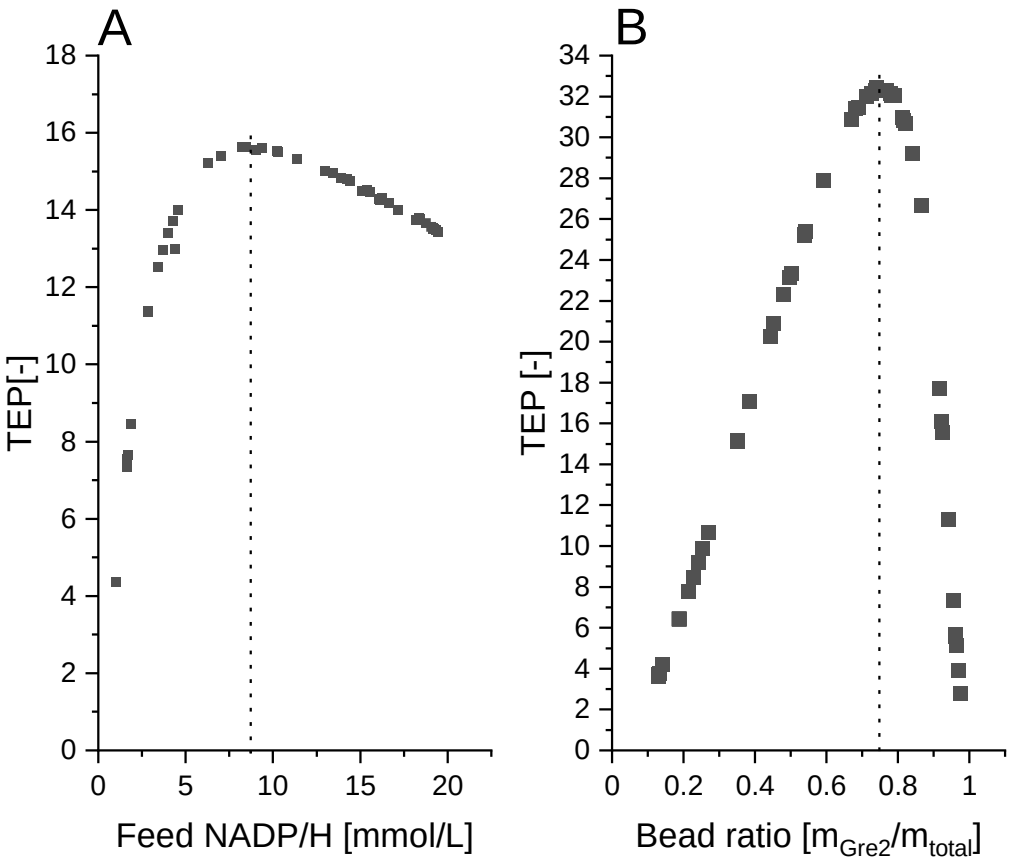

Figure S3 Genetic algorithm results for NADP/H concentration for case 1.3 (A) and bead ratio for case 1.2 (B). Dashed lines indicate the parameter values for maximal TEP for the respective case.

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