Design of Enzymatic Cascade Reactors through Multi-Objective Dynamic Optimization

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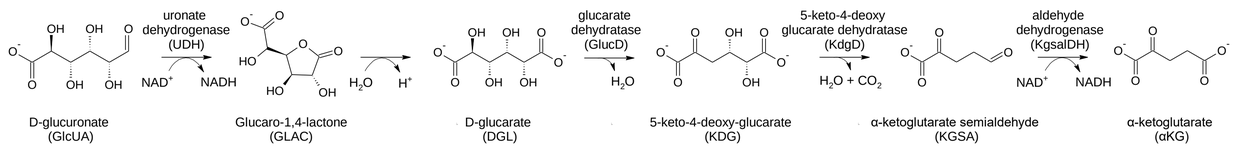
Introduction

Systems with enzymatic cascade reactions are promising alternatives to complex chemical syntheses or microbial bioreactors. This work focusses on the production of α-ketoglutarate (aKG) using two different enzymatic cascade pathways. A novel methodology to scale the cascades from laboratory to production scale and optimize the process parameters is presented. Starting point are kinetic models of the enzyme cascades [1,2] which are combined with a reactor model to a dynamic model of the process. The optimal design of reaction processes is a multi-objective optimization problem [3], in which space-time yield has to be maximized while enzyme consumption rate and cofactor consumption have to be minimized. Pareto-optimal process schedules, which are best compromises between the objectives, are calculated using multi-objective dynamic optimization.

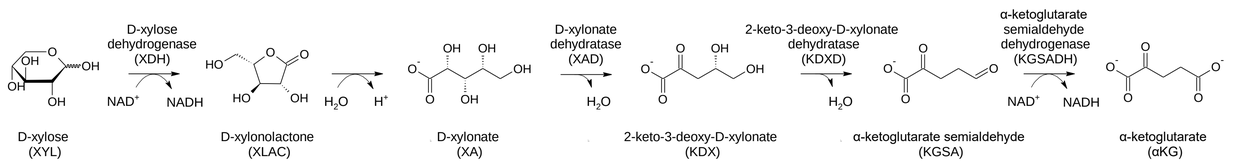
Enzymatic cascades

Two different enzyme cascade pathways for the production of α-ketoglutarate (aKG) are considered:

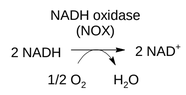
First pathway [1]



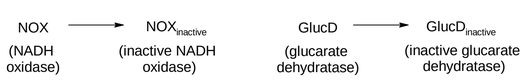
Second pathway [2]



Cofactor regeneration method [4]



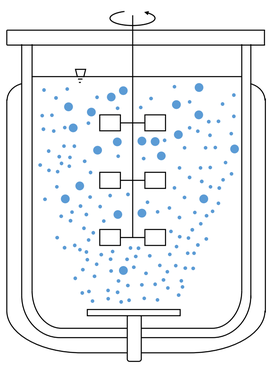
Deactivation of NOX and GlucD [1]



Methodology

Reactor

- Stirred-tank with air gassing [5].
  Two scenarios {k_La = 1.2 min^{-1} and k_La = 15 min^{-1}}.
- Isothermal operation (25°C).
- Initial solution contains cofactor and the first substrate.
- Optimal, time-variant dosing of enzymes.
- Variable Batch time t_f (+ 30 min preparation time).
- The enzymes are not recovered.
- Constraints on yield (yield ≥ 95%).
- Constraints on the concentrations due to solubility.



Optimization objectives:

- Maximize Space-Time Yield (STY).
- Minimize Enzyme Consumption Rate (ECR).
- Minimize Cofactor Concentration (CFC).

Optimization parameters:

- Initial enzyme amount.
- Initial cofactor amount.
- Initial substrate amount.
- Time-variant dosing of enzymes.
- Batch time.

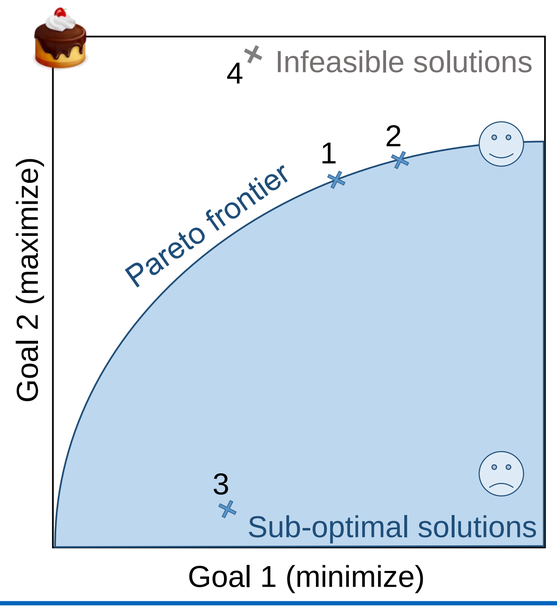
Model

- Michaelis-Menten kinetics adopted from [1], [2].
- First order deactivation kinetics of GlucD and NOX [6].
- Oxygen transfer rate [5].
- Differential material balances for all substrates, the cofactor and enzymes.
- Henry's law for oxygen solubility [5].

Multi-objective optimization

A solution is Pareto-optimal if improvement in one goal is only possible by deterioration in at least one other goal. → Pareto-optimal solutions are best compromises.

Visualization of the objective space for 2 objectives is shown to the right: →



To find Pareto-optimal solutions with 3 objectives, the following problem is solved:

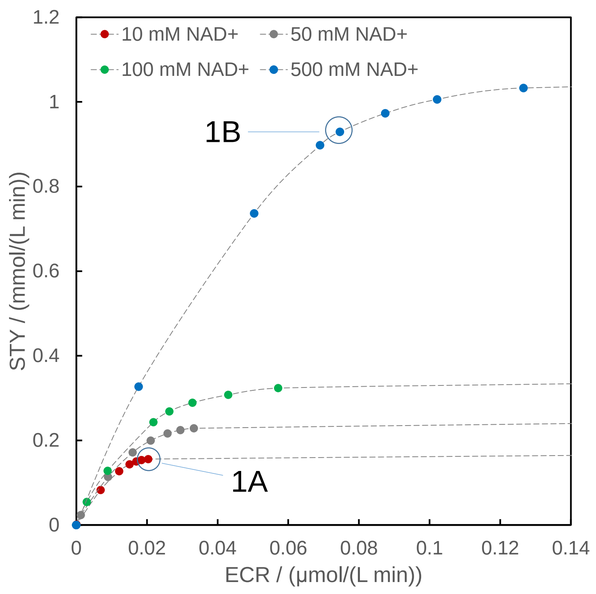
$$\min\ (w \cdot \mathrm{ECR} - (1-w) \cdot \mathrm{STY})$$
$$\text{s.t. } \mathrm{CFC} \le \varepsilon$$

Variation of w and ε yields a set of Pareto-optimal solutions, the Pareto frontiers.

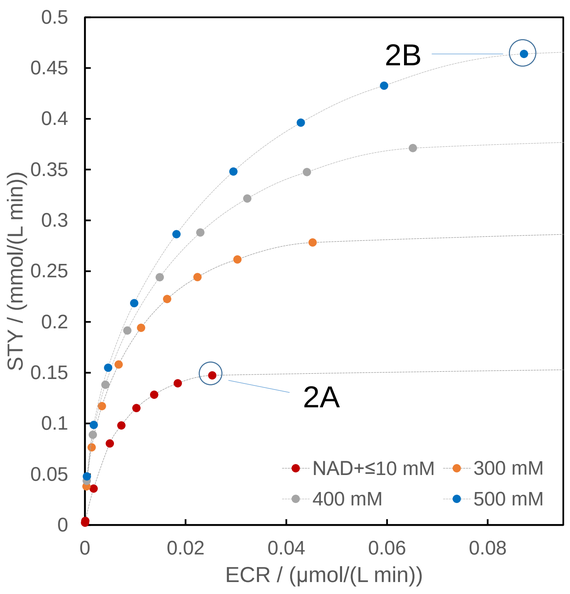
Results

Pareto frontiers and process schedules
Two Pareto frontiers are presented. Each point in the Pareto frontiers corresponds to one optimal process schedule. Selected process schedules are shown in the plots below for the scenario with k_La = 1.2 min^{-1}.

First pathway (k_La = 1.2 min^{-1})

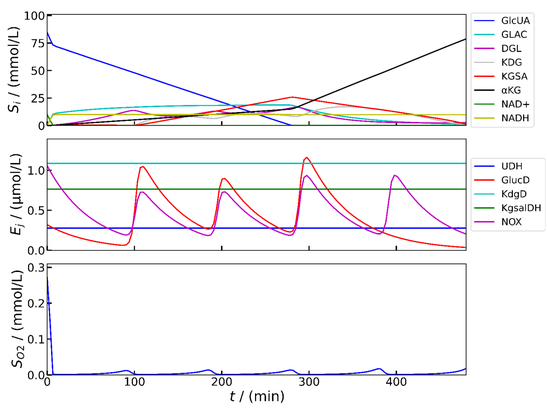


Second pathway (k_La = 1.2 min^{-1})

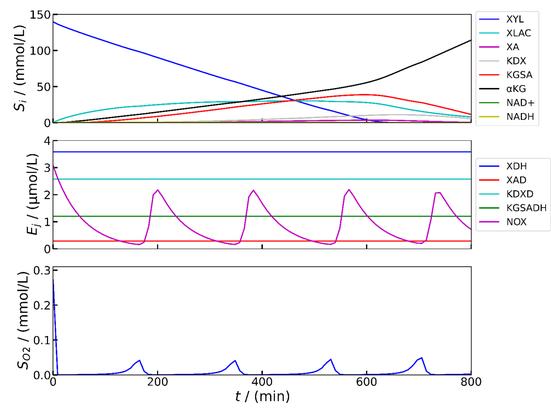


The dashed lines are not quantitative, they are used to visualize how the Pareto frontier extends to the rest of the graph.

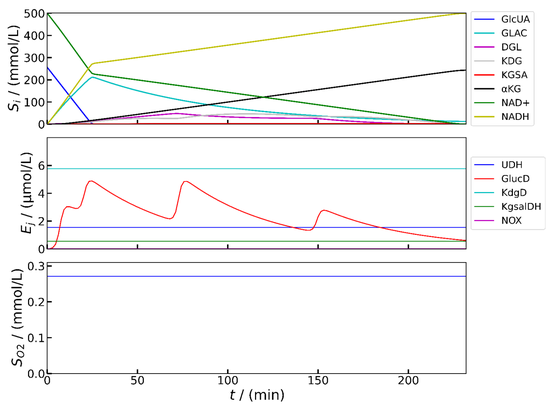
1A: 10 mM initial cofactor concentration, both NOX and GlucD added, oxygen transfer is rate-limiting.



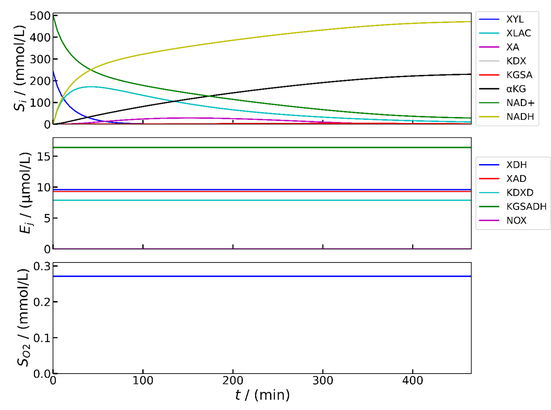
2A: 0.5 mM initial cofactor concentration due to strong inhibition of NAD+ and NADH.



1B: 500 mM initial cofactor concentration, only GlucD is added, the lactone opening (second reaction of the pathway) is rate-limiting.



2B: 500 mM initial cofactor concentration, no NOX is added, the lactone opening (second reaction of the pathway) is rate-limiting.



Contributions

- Dynamic multi-objective optimization was applied to the technical scale-up of two enzyme cascade systems.
- Trade-offs between space-time yield, enzyme consumption rate and cofactor consumption were visualized.
- Results provide decision support for making further experiments, finding bottlenecks in cascades, designing reactors and process schedules.
- Pareto frontiers can be used to compare different enzyme cascades.

References
[1] B. Beer, A. Pick, V. Sieber, Metabolic Engineering 2017, 40, 5-13.
[2] L. Shen, M. Kohlhaas, J. Enoki et al., Nature Communications 2020, 11.
[3] M. Bortz, J. Burger, H. Hasse et al., Computers & Chemical Engineering 2014, 60, 354-363.
[4] M. Sudar, Z. Findrik et al., Biochemical Engineering Journal 2014, 88, 12-18.
[5] P. M. Doran, Bioprocess Engineering Principles, 2013.
[6] Paschalidis et al. (in preparation).

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