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Reactor network synthesis of enzymatic cascades using superstructure optimization

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ABSTRACT

While classical heuristics can be applied to decide on the preferred reactor concept for simple reaction schemes, more complex reaction networks require more sophisticated methods, such as the multilevel reactor design approach or superstructure optimization. Based on an analysis of the existing methods a nonlinear programming framework for a superstructure-based reactor network synthesis is presented, emphasizing numerical robustness and flexible network representation without relying on integer decisions. The approach, which is implemented in GAMS, allows for the combination of continuous stirred-tank and cross-flow reactor models. An exemplary application for the classical Van de Vusse reaction is first shown for validation, prior to the application to an enzymatic cascade based on the Weimberg pathway. Assuming fast co-factor regeneration, the performance of the resulting PFR cascade, which can also be interpreted as a sequence of batch reactions, is compared with a commonly applied single batch reactor. The results show that the two-reactor configuration consistently achieves higher product formation by mitigating inhibition effects, demonstrating the potential of reactor network synthesis for complex reaction systems like enzymatic cascades.

Keywords: reactor network, NLP, GAMS, enzymatic cascades, reaction engineering

INTRODUCTION

The systematic design of reactor networks is a central and enduring challenge in chemical reaction engineering because the selection and interconnection of reactors fundamentally determines achievable yields, selectivities, and overall performance for complex reaction systems. Even when the problem is restricted to kinetics-based descriptions, the number of feasible reactor configurations grows rapidly with increasing system complexity, rendering the use of heuristics or intuitive or case-specific design approaches insufficient and highlighting the need for systematic, theoretically grounded methodologies for reactor network synthesis.

A unifying framework for assessing the limits of reactor performance is provided by the concept of the attainable region (AR), first introduced by Horn [1] as the “totality of physically possible reactors.” The AR represents the complete set of achievable outlet states for a given reaction system under admissible operating conditions, enabling the identification of optimal reactor configurations through geometric arguments. Building on this concept, Feinberg and Hildebrandt [2, 3] demonstrated that the complete attainable region can be constructed from a finite set of elementary reactor building blocks—namely the continuously stirred tank reactor (CSTR), the plug-flow reactor (PFR), and the differential side stream reactor (DSR)—in combination with ideal mixers and splitters. A key implication of this result is that any optimal reactor performance can, in principle, be realized by a reactor network composed exclusively of these elementary units [4].

Despite its conceptual power, the explicit construction of the full attainable region is computationally demanding and becomes impractical for systems of realistic dimensionality. Consequently, over the past four decades, numerous optimization-based targeting methods have been developed to identify optimal or near-optimal reactor configurations without explicitly constructing the entire AR. These approaches follow two different lines,

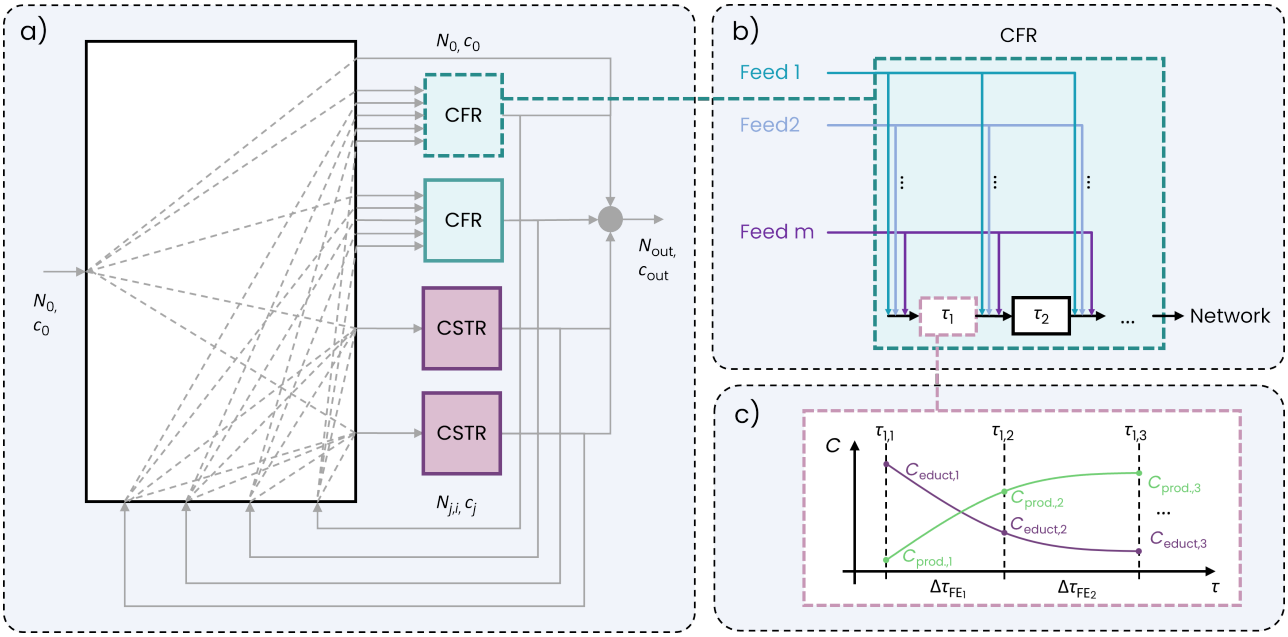

Figure 1. a) An example of the superstructure network for a network with 2 CFRs and 2 CSTRs. b) The connections of the multiple inlet streams inside the CFRs. c) The collocation method for each segment of the CFR (FE stands for finite element).

either pursuing a dynamic optimization of e.g. elementary process functions (EPF) [5], subsequently analyzing the result in a multilevel reactor design approach [6], or pursuing a superstructure-based optimization to determine the optimal reactor network. The latter typically formulate the reactor network synthesis problem as a nonlinear (NLP) or mixed-integer nonlinear programming (MINLP) problem. Examples include discretized recycle reactor modules that interpolate between PFR and CSTR behavior [7], combinations of CSTRs and PFRs [8, 9], networks of just CSTRs [10] and networks composed of CSTRs and DSRs with optional side streams [4, 11]. To render these models tractable, governing differential equations are transformed into algebraic equation systems, either by discretizing PFRs and DSRs as series of CSTRs [8, 12] or by employing direct transcription techniques such as orthogonal collocation on finite elements [4]. The resulting nonlinear and mixed-integer optimization problems are typically solved using local deterministic methods or stochastic metaheuristics, such as genetic algorithms, each with their own limitations in reliability and scalability.

The current contribution revisits the reactor network synthesis problem from both a modeling and numerical robustness perspective and proposes a refined superstructure model that:

- avoids unnecessary integer decisions while retaining the generality needed to capture arbitrary network connectivity,
- relies on continuous stirred tank and cross-flow reactors, and
- is solved by means of a polylithic solution approach in GAMS.

A key novelty of our work is that we demonstrate how many numerical difficulties traditionally associated with reactor network synthesis are not inherent to the problem itself but are instead induced by specific modeling choices.

To validate the proposed methodology and benchmark its performance against established approaches, we first illustrate the application to the classical Van de Vusse reaction network [14] that has been extensively investigated for reactor network synthesis and attainable region analyses.

As a second innovative application, we consider the design of an optimal reactor network for an enzymatic cascade. Such cascades involve a sequence of biocatalytic transformations catalyzed by multiple enzymes. They are of growing interest in biocatalysis and process intensification due to their potential for high selectivity and environmentally friendly operation. One example that is considered in the current study, such as the oxidative Weimberg pathway for pentose conversion to α-ketoglutarate [14], which builds on recent advances in in-vitro enzyme cascade design for metabolic pathways. Up to today, model-based optimization methods for enzymatic cascade reaction systems focus on optimizing the single pot approach [15-18]. Even though, these multi-step reactions can often be carried out in a single pot, this is not

necessarily optimal under all conditions. Inhibition or allosteric effects are only two possible reasons a more complex reactor network can be a beneficial choice. These systems provide an excellent application for reactor network synthesis methods because they combine strongly nonlinear kinetics with interlinked reaction steps.

METHODOLOGY

Model formulation

Figure 1 provides an illustration of the superstructure model used in this work. A generic reactor network superstructure following the state-space approach is formulated to allow arbitrary interconnections between feed streams, reactors, bypasses, and outlet streams via a distribution network. The individual reactors in the network are assumed to operate at steady state. An overall molar balance is imposed over the complete reactor network, ensuring consistency between all feed, reactor, bypass, and outlet flows. Unlike the work of Recker et al. [10], which considers solely ideal CSTRs, also tubular cross flow reactors (CFRs), which can implement a PFR or DSR [11] are considered as building blocks. The system of differential equations for the CFRs is modeled via a direct transcription using orthogonal collocation on finite elements, resulting in a NLP formulation. Intermediate feeding is feasible in between the finite elements, while a Radau IIA collocation captures axial variations in composition along the length of each finite element. For CSTR-only models' large cascades of CSTRs are required to accurately approximate the PFR performance. For kinetically sensitive systems, minor changes in residence time distribution and mixing assumptions can induce disproportionate deviations in the state trajectory, while networks with a large number of CSTRs pose combinatorial challenges for optimization. With the combination of CSTRs and CFRs a superstructure with two of these reactors is rich enough for most applications.

Unlike the EPF-approach that requires an interpretation of internal fluxes to identify CSTRs [19], the superstructure implements CSTRs as perfectly mixed reactors, with outlet compositions determined by the steady-state reaction rates and an explicit residence time. The superstructure formulation allows flexible stream splitting, mixing, and recycling, enabling the optimization of network topology, reactor selection, and sizing, while ensuring that material balances and component mole fractions are satisfied throughout the network.

Model implementation

The superstructure model is implemented in GAMS. A polylithic modeling and solution strategy is employed, which starts with the definition of the reaction kinetics that are included for each reactor type, with the flexibility to incorporate multiple reaction mechanisms. On this basis each individual reactor is initialized based on the overall feed and a small residence time. After the definition of potential additional constraints, e.g. for the definition of a minimum conversion or an upper bound for some intermediate composition, an initial solution for the full superstructure model is determined for a distribution network with a fixed structure that distributes the feed to all available reactors and does the same for the reactor products. After this initialization, the fixed split factors in the distribution network are relaxed and the reactor network optimization is performed.

Optimization of the superstructure is performed using nonlinear programming solvers (e.g., CONOPT), maximizing a specified performance objective, while satisfying all reactor and network constraints. Note that solver options, scaling factors, and finite-element discretization have to be selected to ensure numerical stability depending on the specific application. The respective model implementations for the subsequent case studies are provided via Gitlab (link in the appendix).

CASE STUDIES

Van de Vusse reaction

A commonly studied example for reactor network optimization is the so-called Van de Vusse reaction shown in Figure 2:

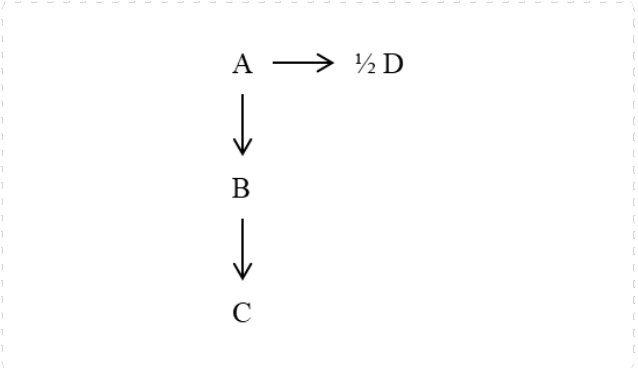

Figure 2. The chemical reaction network of the widely studied Van de Vusse reaction.

All reactions are catalyzed by the same catalyst. The kinetic and feed-related parameters are reflecting the case study 1 considered in the work of [19]. The Yield of B is selected as an objective to be maximized by selecting the specific reactor network structure and the residence time and temperature of the isothermal reactors.

Weimberg pathway

Reactor network synthesis is particularly interesting for applications to enzymatic cascades, as they involve multiple enzymes whose concentrations must be carefully balanced to achieve optimal performance. The

reaction network of the Weimberg pathway is shown in Figure 3. This cascade is well characterized kinetically, with each step catalyzed by a distinct enzyme. Two reactions in the pathway are cofactor dependent.

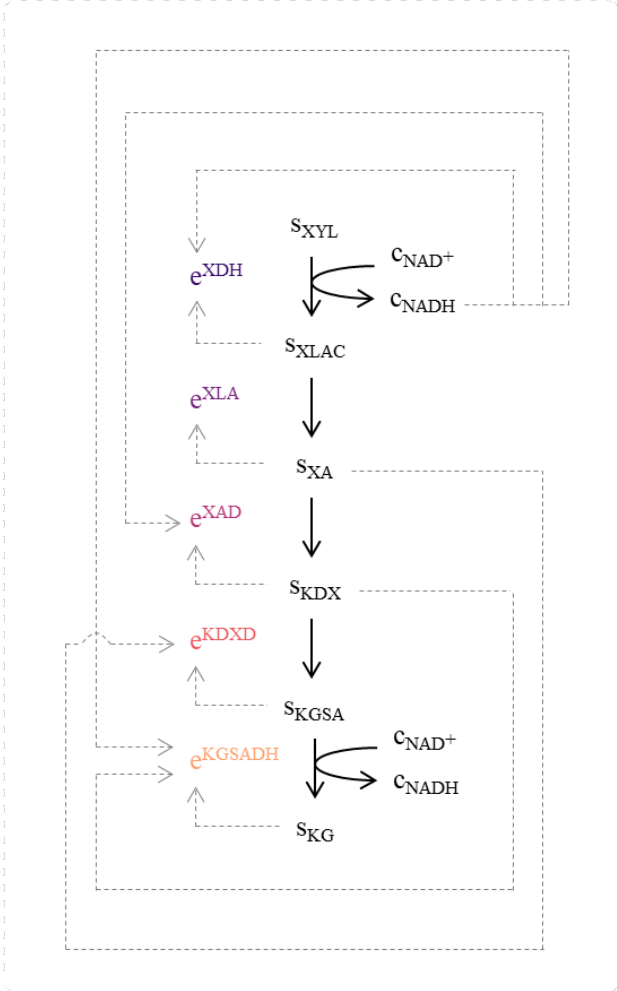

Figure 3: The chemical reaction network of the enzymatic cascade reaction of the Weimberg pathway. The black arrors show the reaction steps, while the grey, dashed arrows show inhibition effects.

Figure 3. also presents the substrate (s) and enzyme (e) abbreviations, together with the chemical reaction network and known inhibitory interactions between substrates and enzymes (dashed lines). A detailed kinetic model of the Weimberg pathway was developed by Shen et al. [14]. In their study, cofactor regeneration was implemented via a secondary reaction converting pyruvate to L-lactate catalyzed by L-lactate dehydrogenase. Under the assumption that this regeneration step proceeds rapidly—corresponding to the use of sufficiently high L-lactate dehydrogenase levels—we apply the proposed methodology to evaluate the performance gains achievable by employing multiple batch reactors instead of a single batch reactor for the Weimberg pathway. The well-known analogy between batch reactors and plug-flow reactors was used to apply our steady-state centric methodology to this dynamic problem.

The objective of the optimization problem is to maximize product formation (s_{KG}). CONOPT3 is employed as NLP solver in GAMS analyzing two scenarios: (i) a single-reactor configuration, in which the optimizer determines the optimal enzyme titers, and (ii) a two-reactor configuration in series, in which the optimizer determines the optimal allocation of a fixed enzyme budget between the reactors. The GAMS models are available in a GitLab repository (link provided in the appendix).

RESULTS AND DISCUSSION

Van de Vusse reaction

To validate the reactor network synthesis approach, the case study with parameters considered by Xie et al. [19] was solved. The resulting optimal reactor network matched the previously reported configuration, consisting of a CSTR followed by a PFR in series (cf. Figure 4), which also reflects the objective function and design variables reported by Xie et al [19] (objective function value: $Y_{A\rightarrow B} = 63.5\ \%$).

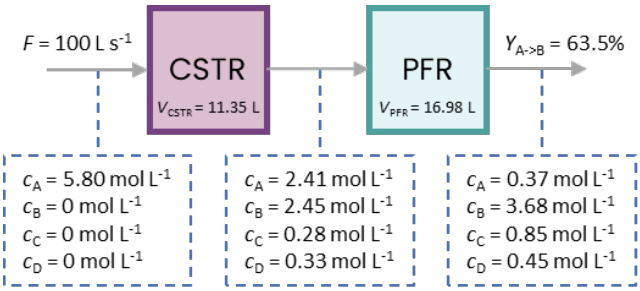

Figure 4. The results of the Van de Vusse case study.

Optimization of the Van de Vusse case study also allows for the identification of some hidden pitfalls for the flexible CFR model. If side-feeding and side-draws locations are feasible and the residence time in each finite element is treated as decision variable the solver exploits this by activating feed injections and effluent withdrawal at intermediate locations, effectively bypassing finite elements and reducing the numerical accuracy of the integration of the differential equation system. This concentrates residence time only in the most sensitive segments, resulting in a degenerate optimum: comparable objectives obtained with different apparent reactor sizes and partial volume utilization, which is not a faithful realization of the intended CFR behavior. Consequently, the different degrees of freedom in the CFR model should be used with care, to avoid such compromising bypass streams.

Weimberg pathway

The results of the comparison between the single batch reactor and the superstructure with two PFR (batch reactors) are summarized in Figure 5. The x-axis represents the total enzyme loading, while the y-axis denotes the total substrate concentration, for which each 3 distinct values have been evaluated. The percentage improvement in product formation achieved by the resulting two-reactor configuration is shown for each combination as a third dimension in the heat-map representation.

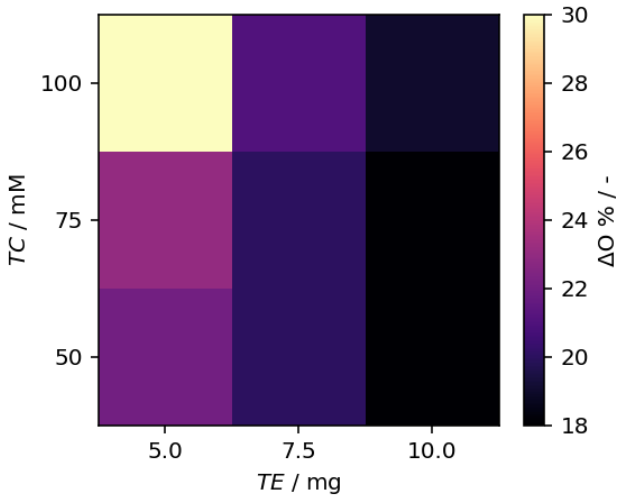

Figure 5. Comparison of the produced product with two batch reactors against the produced product with one reactor for different substrate and enzyme amounts in a raster-type contour plot.

For all conditions, the resulting two-reactor network consistently outperforms the single-reactor configuration, with up to 30% more product with the same enzyme budget. The performance advantage becomes increasingly pronounced at higher substrate loadings and lower total enzyme amounts.

Further insight is provided by examining the enzyme allocation strategy identified by the optimizer. Figure 6 illustrates the optimal enzyme distributions for the case of 75 mM substrate and 7.5 mg total enzyme. In addition to determining the optimal relative enzyme ratios, the optimizer also identifies the optimal partitioning of enzymes between the reactors when the two-reactor network is considered.

The optimizer preferentially distributes enzymes between the two reactors to mitigate inhibitory effects, particularly those affecting the final two enzymes in the cascade. This result is noteworthy, as it challenges the prevailing assumption in enzymatic cascade design that co-localizing all enzymes within a single reactor is invariably optimal.

CONCLUSIONS

A continuous NLP-based framework for reactor network synthesis was presented and demonstrated on both classical and biocatalytic reaction systems. By avoiding integer decisions and relying on carefully formulated CSTR and CFR models, the approach enables the systematic identification of optimal reactor configurations while maintaining numerical robustness.

Application to the Weimberg enzymatic cascade showed that splitting the reaction across two batch reactors can provide substantial kinetic advantages over conventional single-pot operation. The optimized solutions consistently favored enzyme distributions that alleviate inhibition effects, particularly in the downstream cascade steps, resulting in higher product formation under constrained enzyme budgets. This behavior challenges the commonly held assumption that enzymatic cascades should always be operated in a single reactor and highlights the importance of considering reactor network structure as an additional design degree of freedom.

Overall, the results demonstrate that reactor network synthesis can provide valuable insights into both achievable performance limits and optimal process configurations for complex reaction systems. When combined with careful modeling choices, the proposed framework offers a reliable and flexible tool for the design of advanced reactor networks in both chemical and biochemical applications.

DIGITAL SUPPLEMENTARY MATERIAL

The GAMS code used in this article is given in the following repository.

https://collaborating.tuhh.de/clp3832/escape-36-reactor-network-synthesis-of-enzymatic-cascades-using-superstructure-optimization/-/tree/b48a99412abe854ae54642805688e7451c7a8eff/

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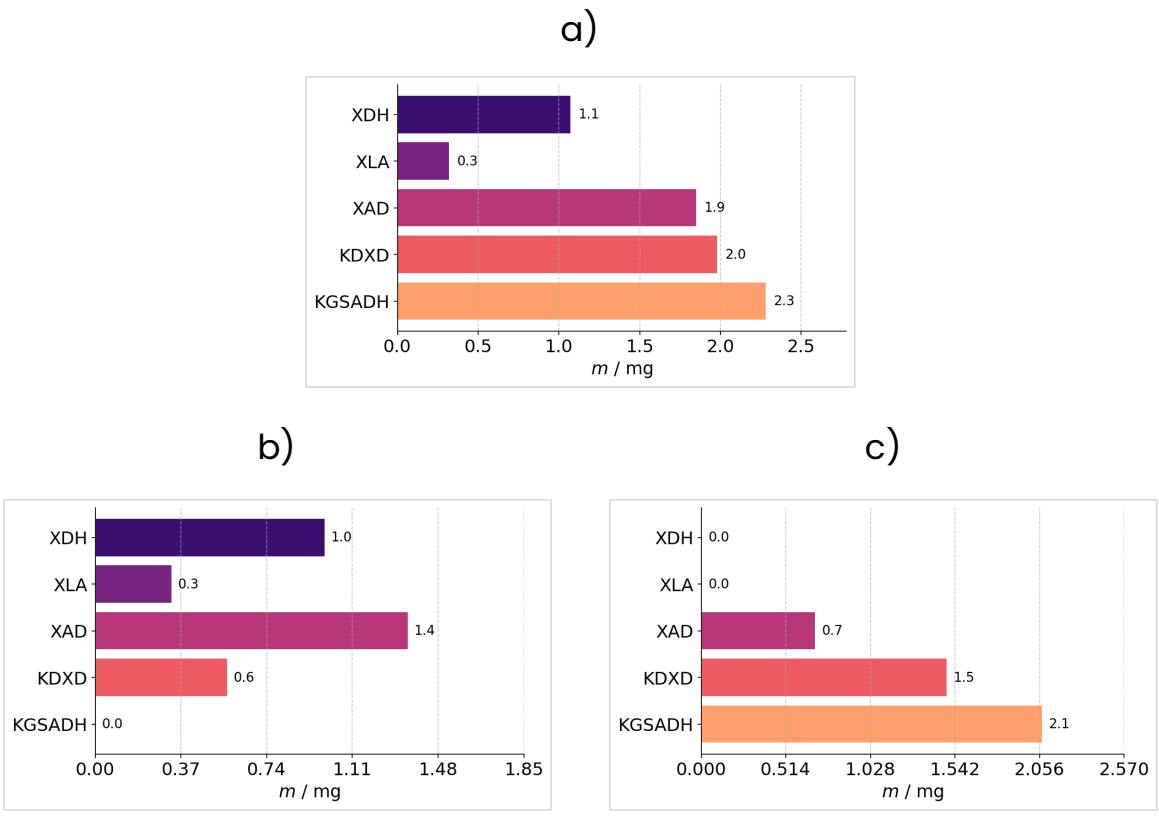

Figure 6. a) The suggested enzyme ratios in the single reactor network. b) The suggested enzyme ratios in the first reactor of the two reactor network. c) The suggested enzyme ratios in the second reactor of the two reactor network.

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REFERENCES

1. G. Horn, “Attainable and Non-Attainable Regions in Chemical Reaction Technique, ” Proc. Eur. Symp. on Chemical Reaction Engineering, Pergamon Press, London, 1964.
2. Feinberg M. Toward a theory of process synthesis. Ind. Eng. Chem. Res. 41:3751-3761 (2002). https://doi.org/10.1021/ie010807f
3. Hildebrandt D, Glasser D. The attainable region and optimal reactor structures. Chemical Engineering Science 45:2161-2168 (1990). https://doi.org/10.1016/0009-2509(90)80091-r
4. Lakshmanan A, Biegler LT. Synthesis of optimal chemical reactor networks. Ind. Eng. Chem. Res. 35:1344-1353 (1996). https://doi.org/10.1021/ie950344b
5. Freund H, Sundmacher K. Towards a methodology for the systematic analysis and design of efficient chemical processes. Chemical Engineering and Processing: Process Intensification 47:2051-2060 (2008). https://doi.org/10.1016/j.cep.2008.07.011
6. Freund H, Maußner J, Kaiser M, Xie M. Process intensification by model-based design of tailor-made reactors. Current Opinion in Chemical Engineering 26:46-57 (2019). https://doi.org/10.1016/j.coche.2019.08.003
7. L. E. K. Achenie and L. T. Biegler, “Algorithmic synthesis of chemical reactor networks using mathematical programming, ” Ind. Eng. Chem. Fundam., vol. 25, no. 4, pp. 621–627, 1986.
8. Gholizadeh Z, Soltani H, Javid M, Azar MS. A new robust approach for reactor network synthesis by combination of mathematical method and NSGAII. International Journal of Chemical Reactor Engineering 18: (2020). https://doi.org/10.1515/ijcre-2019-0090
9. Wen Y, Biegler LT, Ochoa MP, Matthews L, Ferrio J, Weston J, Nikbin N. Continuous reactor network design for rigid polyol production. Chemical Engineering Science 230:116189 (2021).

https://doi.org/10.1016/j.ces.2020.116189
10. Recker S, Skiborowski M, Redepenning C, Marquardt W. A unifying framework for optimization-based design of integrated reaction–separation processes. Computers & Chemical Engineering 81:260-271 (2015). https://doi.org/10.1016/j.compchemeng.2015.03.014
11. Schweiger CA, Floudas CA. Synthesis of optimal chemical reactor networks. Computers & Chemical Engineering 23:S47-S50 (1999). https://doi.org/10.1016/s0098-1354(99)80013-1
12. Marcoulaki E, Linke P, Kokossis A. Design of separation trains and reaction-separation networks using stochastic optimization methods. Chemical Engineering Research and Design 79:25-32 (2001). https://doi.org/10.1205/026387601528499
13. Biki? D, Butinar B, Glavi? P. Optimal reactor systems for van de vusse reaction scheme with multicomponent feed. Computers & Chemical Engineering 26:1335-1343 (2002). https://doi.org/10.1016/s0098-1354(01)00759-1
14. Shen L, Kohlhaas M, Enoki J, Meier R, Schönenberger B, Wohlgemuth R, Kourist R, Niemeyer F, van Niekerk D, Bräsen C, Niemeyer J, Snoep J, Siebers B. A combined experimental and modelling approach for the weimberg pathway optimisation. Nat Commun 11: (2020). https://doi.org/10.1038/s41467-020-14830-y
15. Paschalidis L, Beer B, Sutiono S, Sieber V, Burger J. Design of enzymatic cascade reactors through multi-objective dynamic optimization. Biochemical Engineering Journal 181:108384 (2022). https://doi.org/10.1016/j.bej.2022.108384
16. L. Paschalidis, D. Fröschl, M. Ibañez, S. Sutiono, V. Sieber, and J. Burger, “Boosting of enzymatic cascades by intermediates: theoretical analysis and model-based optimization, ” Biochem. Eng. J., p. 109440, 2024.
17. Paschalidis L, Arana?Peña S, Sieber V, Burger J. Modeling enzymatic cascade reactions immobilized in plug?flow reactors for flow biocatalysis. Chemie Ingenieur Technik 96:741-748 (2024). https://doi.org/10.1002/cite.202300159
18. Milker S, Fink MJ, Oberleitner N, Ressmann AK, Bornscheuer UT, Mihovilovic MD, Rudroff F. Kinetic modeling of an enzymatic redox cascade in vivo reveals bottlenecks caused by cofactors. ChemCatChem 9:3420-3427 (2017). https://doi.org/10.1002/cctc.201700573
19. Xie M, Freund H. Fast synthesis of optimal chemical reactor networks based on a universal system representation. Chemical Engineering and Processing - Process Intensification 123:280-290 (2018). https://doi.org/10.1016/j.cep.2017.11.011