Application of Iterative Robust Model-based Optimal Experimental Design for the Calibration of Biocatalytic Models

The aim of model calibration is to estimate unique parameter values from available experimental data, here applied to a biocatalytic process. The traditional approach of first gathering data followed by performing a model calibration is inefficient, since the information gathered during experimentation is not actively used to optimise the experimental design. By applying an iterative robust model-based optimal experimental design, the limited amount of data collected is used to design additional informative experiments. The algorithm is used here to calibrate the initial reaction rate of an ω-transaminase catalysed reaction in a more accurate way. The parameter confidence region estimated from the Fisher Information Matrix is compared with the likelihood confidence region, which is a more accurate, but also a computationally more expensive method. As a result, an important deviation between both approaches is found, confirming that linearisation methods should be applied with care for nonlinear models. This article is protected by copyright. All rights reserved.

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Automated Determination of Oxygen-Dependent Enzyme Kinetics in a Tube-in-Tube Flow Reactor

Enzyme-mediated oxidation is of particular interest to synthetic organic chemists. However, the implementation of such systems demands knowledge of enzyme kinetics. Conventionally collecting kinetic data for biocatalytic oxidations is fraught with difficulties such as low oxygen solubility in water and limited oxygen supply. Here, we present a novel method for the collection of such kinetic data using a pressurized tube-in-tube reactor, operated in the low-dispersed flow regime to generate time-series data, with minimal material consumption. Experimental development and validation of the instrument revealed not only the high degree of accuracy of the kinetic data obtained, but also the necessity of making measurements in this way to enable the accurate evaluation of high $K_{MO}$ enzyme systems. For the first time, this paves the way to integrate kinetic data into the protein engineering cycle.

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Scopus rating (2014): CiteScore 4.52 SJR 1.89 SNIP 1.103
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ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
Scopus rating (2012): CiteScore 4.58 SJR 2.394 SNIP 1.141
Web of Science (2012): Impact factor 5.181
ISI indexed (2012): ISI indexed yes
Web of Science (2012): Indexed yes
Scopus rating (2011): CiteScore 4.3 SJR 2.229 SNIP 1.069
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Web of Science (2010): Indexed yes
Development of in-situ product removal strategies in biocatalysis applying scaled-down unit operations

An experimental platform based on scaled-down unit operations combined in a plug-and-play manner enables easy and highly flexible testing of advanced biocatalytic process options such as in-situ product removal (ISPR) process strategies. In such a platform it is possible to compartmentalize different process steps while operating it as a combined system, giving the possibility to test and characterize the performance of novel process concepts and biocatalysts with minimal influence of inhibitory products. Here the capabilities of performing process development by applying scaled-down unit operations are highlighted through a case study investigating the asymmetric synthesis of 1-methyl-3-phenylpropylamine (MPPA) using ω-transaminase, an enzyme in the sub-family of amino transferases (ATAs). An on-line HPLC system was applied to avoid manual sample handling and to semi-automatically characterize ω-transaminases in a scaled-down packed-bed reactor (PBR) module, showing MPPA as a strong inhibitor. To overcome the inhibition, a two-step liquid-liquid extraction (LLE) ISPR concept was tested using scaled-down unit operations combined in a plug-and-play manner. Through the tested ISPR concept, it was possible to continuously feed the main substrate benzylacetone (BA) and extract the main product MPPA throughout the reaction, thereby overcoming the challenges of low substrate solubility and product inhibition. The tested ISPR concept achieved a product concentration of 26.5 g MPPA·L⁻¹, a purity up to 70% g MPPA·L⁻¹ and a recovery in the range of 80% mol·mol⁻¹ of MPPA in 20 hours, with the possibility to increase the concentration, purity and recovery further. This article is protected by copyright. All rights reserved.

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Scopus rating (2015): CiteScore 4.44 SJR 1.632 SNIP 1.355
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BFI (2014): BFI-level 1
Scopus rating (2014): CiteScore 4.16 SJR 1.612 SNIP 1.395
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Web of Science (2014): Indexed yes
BFI (2013): BFI-level 2
Scopus rating (2013): CiteScore 4.44 SJR 1.637 SNIP 1.427
Web of Science (2013): Impact factor 4.164
A case study on robust optimal experimental design for model calibration of ω-Transaminase

Proper calibration of models describing enzyme kinetics can be quite challenging. This is especially the case for more complex models like transaminase models (Shin and Kim, 1998). The latter fitted model parameters, but the confidence on the parameter estimation was not derived. Hence, the usability of the parameter estimates is difficult to assess. In this paper, the confidence is derived, using the Fisher Information Matrix (FIM) for the backward reaction (conversion of acetophenone and alanine to α-methylbenzylamine and pyruvate). FIM computation requires local parameter sensitivities...
and measurement errors. Since the latter was not provided, a conservative standard deviation of 5% was assumed. The confidence analysis yielded that only two (Vr and Kac) out of five parameters were reliable estimates, which means that model predictions and decisions based on them are highly uncertain. The reason behind this problem is practical identifiability, which can be related to both the model structure and/or the information content of the data. The available data are 25 experiments performed by Shin and Kim, set up in a 5x5 factorial design (2 substrates with 5 concentration levels each) across the experimental space. However, it is expected that more informative experiments can be designed to increase the confidence of the parameter estimates. Therefore, we apply Optimal Experimental Design (OED) to the calibrated model of Shin and Kim (1998). The total number of samples was retained to allow fair comparison with the original experimental design. Using OED led to unique and higher quality parameter estimates for all parameters. This illustrates that OED can increase parameter confidence without increasing the experimental effort. The main problem which arises when performing OED is that the "real" parameter values are not known before finishing the model calibration. However, it is important that the chosen parameter values are close to the real parameter values, otherwise the OED can possibly yield non-informative experiments. To counter this problem, one can use robust OED. The idea of robust OED is to make the design less dependent on one specific parameter set, but make it suitable for a subset of parameters in a local parameter space. This robust OED methodology is currently being applied to the backward part of the model of Shin and Kim (1998) to design experiments for the conversion of 1-methyl-2-phenylpropylamine and acetone to benzylacetone and isopropylamine and yield a reliable estimation for all parameters. Details of the outcome will be shown at the conference.

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A microfluidic toolbox for the development of in-situ product removal strategies in biocatalysis
A microfluidic toolbox for accelerated development of biocatalytic processes has great potential. This is especially the case for the development of advanced biocatalytic process concepts, where reactors and product separation methods are closely linked together to intensify the process performance, e.g., by the use of in-situ product removal (ISPR). This review provides a general overview of currently available tools in a microfluidic toolbox and how this toolbox can be applied to the development of advanced biocatalytic process concepts. Emphasis is placed on describing the possibilities and advantages of the microfluidic toolbox that are difficult to achieve with conventional batch-process-based technologies. Application of this microfluidic toolbox will potentially make it possible to intensify biocatalytic reactions and thereby facilitate the development towards novel and advanced biocatalytic processes, which in many cases have proven too difficult in conventional batch equipment.

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Web of Science (2016): Impact factor 1.768
Enzyme Characterization in Microreactors by UV-Vis Spectroscopy

In protein engineering mutants are often selected solely on the basis of activity [1], simplifying the analysis and enabling high throughput screening. At a later stage of development, several mutants show comparable performance and this basis for selection becomes indistinct. The basis for selection can at this point be improved by characterization of the enzyme performance where also inhibition and toxicity effects are taken into account. Enzyme characterization is here defined as the effect on initial rate of reaction with respect to pH, enzyme, substrate, co-substrate, product and co-product concentration [2]. From this investigation, it will be possible to determine whether the enzyme meets the criteria for process requirements or not. The development of the process will determine the requirements and this can also reach a state of maturity that resolves obstacles, lowers criteria and paves the way for implementation. As an example ω-transaminase is here investigated, which facilitates the exchange of an amine- and keto-group stereoselectively. The characterization will be carried out in a microreactor [3], this size is currently the only concept that can facilitate this thorough analysis, as the enzyme resource is scarce at this point of development. In the case where the reaction operates with UV active components, UV can be used to detect compounds with high sensitivity supplemented by multivariate data analysis. The spectra are here decorrelated and regressed to yield concentrations of individual compounds. HPLC systems are built for handling small quantities of liquids and the UV detectors for these proves to be fitting excellent. Enzyme characterization is therefore carried out by a combination of a microreactor with a diode array detector from an HPLC system.

The application of reaction engineering to biocatalysis

Biocatalysis is a growing area of synthetic and process chemistry with the ability to deliver not only improved processes for the synthesis of existing compounds, but also new routes to new compounds. In order to assess the many options and strategies available to an engineer developing a new biocatalytic process, it is essential to carry out a systematic evaluation to progress rapidly and ensure decisions are made on firm foundations. In this way, directed development can be carried out and the chances of implementation of a commercially successful process can be much improved. In this review we outline the benefits of reaction engineering in this development process, with particular emphasis of reaction kinetics. Future research needs to focus on rapid methods to collect such data at sufficient accuracy that it can be used for the effective design of new biocatalytic processes.
Application of A Microfluidic Tool for the Determination of Enzyme Kinetics

Biocatalysis offers the ability to carry out important synthesis and production of valuable chemicals at benign conditions. In the development of new processes, enzymes are being engineered towards specific products with great success. Currently, mutations are introduced into enzymes, and mutants are formed thereof and a search among these is conducted. High throughput screening can deliver screening of mutants in the order of millions a day. Enzyme mutants with increased performance are therefore likely to be found. Here, the enzyme amine transaminases is evaluated since it offers a unique way of producing chiral amines. These amines are important as building blocks for pharmaceuticals and agrochemicals. A promising enzyme has been found, but it has been a problem to assess its performance and give process development direction. Common limitations are substrate and product solubility, unfavourable thermodynamics, inhibition and stability. It is a difficult task to assess where the current bottle neck is for a desired process. Moreover, it cannot be expected that a single solution to the limitations can be found and rather an integrated solution of all of the problems should be the future aim. All the limitations surround the reactor of a process, and with the performance of this being unknown, it is almost impossible to direct development. A focal point must therefore lie in the determination of kinetic models and how kinetic data can be obtained in a robust and generic way. Models for many enzymes already exist and can be found in common text books. These models do however require mutant specific data and must be collected with the target reaction. In this thesis a novel way of collecting kinetic data is created, this is carried out by combining existing technology and enables the analysis of aqueous solutions on-line. Furthermore, the use of a size exclusion column enables the simultaneous detection of enzymes and UV/VISactive compounds. The size exclusion chromatography does not provide baseline separated results, nor is this required. The application of chemometric tools enable detection of compounds in the collected retention time wavelength data. A major improvement over traditional techniques is the quantification of enzyme concentration and this makes it possible to use specific activities for model fitting. The setup takes advantage of microfluidic features and delivers semi-automatic experimentation, overall reducing both consumption of precious materials and costly labor.
Synthesis of 5-hydroxymethylfurfural (HMF) by acid catalyzed dehydration of glucose-fructose mixtures

Synthesis of 5-hydroxymethylfurfural (HMF) from hexoses has been studied extensively in the scientific literature. However, a process has yet to be implemented at industrial scale. In this paper the simultaneous dehydration of glucose and fructose was investigated, in order to develop a process allowing the use of the cheapest available source of fructose: high fructose corn syrup. The dehydration was catalyzed by hydrochloric acid and conducted in acetone-water mixtures, which ensured good selectivity towards HMF and eliminated precipitation of polymer by-products (insoluble humins). Through a detailed experimental investigation a reaction network was proposed, and subsequently the corresponding kinetic model was fitted to experimental data in order to obtain estimates of the reaction kinetic parameters. The kinetic model is capable of predicting the formation of HMF along with the important by-products: soluble humins, glucose dimers, anhydroglucose, and formic acid. The reaction conditions in four different reactor configurations were optimized and compared using the kinetic model. It was found that a recirculating reactor setup is preferable, where the equilibrium controlled by-products (anhydroglucose and glucose dimers) are recirculated to the dehydration reactor. The model predicts an HMF selectivity of close to 70% in a recirculating reactor at conditions where HMF degradation is avoided.

General information
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Organisations: Department of Chemical and Biochemical Engineering, CAPEC-PROCESS, Novozymes A/S
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Scopus rating (2015): CiteScore 5.68
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BFI (2014): BFI-level 2
Scopus rating (2014): CiteScore 4.92
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 1
Scopus rating (2013): CiteScore 4.59
Web of Science (2013): Impact factor 4.058
ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
BFI (2012): BFI-level 1
Scopus rating (2012): CiteScore 3.92
Web of Science (2012): Impact factor 3.473
Biocatalytic process development using microfluidic miniaturized systems
The increasing interest in biocatalytic processes means there is a clear need for a new systematic development paradigm which encompasses both protein engineering and process engineering. This paper argues that through the use of a new microfluidic platform, data can be collected more rapidly and integrated with process modeling, can provide the basis for validating a reduced number of potential processes. The miniaturized platform should use a smaller reagent inventory and make better use of precious biocatalysts. The EC funded BIOINTENSE project will use ω-transaminase based synthesis of chiral amines as a test-bed for assessing the viability of such a high throughput biocatalytic process development, and in this paper, such a vision for the future is presented.

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Enzyme characterisation in microreactors by multivariate data analysis

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Contributors: Ringborg, R. H., Krühne, U., Woodley, J.
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The focus of this work is on process systems engineering (PSE) methods and tools, and especially on how such PSE methods and tools can be used to accelerate and support systematic bioprocess development at a miniature scale. After a short presentation of the PSE methods and the bioprocess development drivers, three case studies are presented. In the first example it is demonstrated how experimental investigations of the bi-enzymatic production of lactobionic acid can be modeled with help of a new mechanistic mathematical model. The reaction was performed at lab scale and the prediction quality analyzed. In the second example a computational fluid dynamic (CFD) model is used to study mass transfer phenomena in a microreactor. In this example the model is not only used to predict the transient dynamics of the reactor system but also to extract material properties like the diffusion velocities of substrate and product, which is otherwise difficult to access. In the last example, a new approach to the design of microbioreactor layouts using topology optimization is presented and discussed. Finally, the PSE methods are carefully discussed with respect to the complexity of the presented approaches, the applicability with respect to practical considerations and the opportunity to analyze experimental results and transfer the knowledge between different scales.

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3D-printer vejen til innovation?

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Applications, benefits and challenges of flow chemistry

Organic synthesis (incorporating both chemo-catalysis and biocatalysis) is essential for the production of a wide range of small-molecule pharmaceuticals. However, traditional production processes are mainly based on batch and semi-batch operating modes, which have disadvantages from an economic, environmental and manufacturing perspective. A potential solution to resolve these issues is to use flow chemistry in such processes, preferably with applications of micro-and mini-sized equipment. In addition, Process Analytical Technology (PAT) may be implemented in a very efficient way in such equipment due to the high degree of automation and process controllability that can be achieved in small scale continuous equipment.

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BFI (2016): BFI-level 1
Scopus rating (2016): CiteScore 0.44 SJR 0.238 SNIP 0.215
Web of Science (2016): Impact factor 0.597
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 1
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Biokatalysatorens vej til industrien

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Miniaturized experimental toolbox for ω-transaminase technology (BIOINTENSE)

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Towards effective biocatalytic process development using microreactor technology

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Using micro technology in process screening for improved ω-transaminases

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**Using micro technology in process screening for improved ω-transaminases**

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**Projects:**

**Mastering bioprocess Integration and Intensification across scales**
Ringborg, R. H., PhD Student, Department of Chemical and Biochemical Engineering
Woodley, J., Main Supervisor, Department of Chemical and Biochemical Engineering
Gernaey, K. V., Supervisor, Department of Chemical and Biochemical Engineering
Krühne, U., Supervisor, Department of Chemical and Biochemical Engineering
Nordblad, M., Examiner, Department of Chemical and Biochemical Engineering
Hessel, V., Examiner
Jensen, K. F., Examiner
1/3 FUU, 1/3 inst 1/3 Andet
01/10/2012 → 02/12/2015
Award relations: Mastering bioprocess Integration and Intensification across scales
Project: PhD