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# Systematic Development of Miniaturized (Bio)Processes using Process Systems Engineering (PSE) Methods and Tools

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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.

*Key words:*

Computational Fluid Dynamics (CFD), modeling, Process Systems Engineering (PSE), (bio)processes

## Introduction

The development of new chemical engineering design tools is essential for the implementation of the latest technology in the manufacture of chemical and other products. The focus of this paper is on process systems engineering (PSE) methods and tools, and especially on how such PSE methods and tools can be applied to speed up or support systematic bioprocess development at miniature scale. In this context, the term bioprocess is interpreted broadly, and includes both biocatalysis (enzyme or resting cell conversion) as well as fermentation (growing cell conversion). In the following section, we first provide a brief introduction to the main drivers of biocatalysis and fermentation process development. The paper also contains a short overview of PSE methods and tools. The use of such tools is illustrated on the basis of three examples, which summarize some of our recent experiences in the area. The paper ends with a discussion on future perspectives with respect to the use of PSE methods and tools in miniaturized bioprocess systems and for extrapolation of results across reactor scales (scaling up).

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### Bioprocess development drivers – biocatalysis

The need for selective chemistry is the main driver behind the increasing academic and industrial interest in biocatalytic processes (chemical reactions catalyzed by an isolated enzyme, immobilized enzyme or whole cell containing one or more enzymes).[1] While biocatalysis may easily hold the promise of high selectivity, economic process feasibility is also necessary for implementation in industry. Economic feasibility translates into a minimum required product concentration that must leave the reactor, as well as a yield of product on biocatalyst that is to be achieved, as has been illustrated by Tufvesson and coworkers for a number of different scenarios.[2] The exact threshold values for minimum product concentration and yield of product on biocatalyst will indeed depend on the particular industry sector as well as the selling cost of the product relative to the cost of the substrate. In fact, most new biocatalytic processes studied in the laboratory do not fulfill these requirements, mainly because enzymes are usually evolved to operate under mild conditions converting natural substrates at low concentrations. Hence, achieving an economically feasible biocatalytic process in terms of minimum re-

quired product concentration and yield of product on biocatalyst is therefore often challenging, and can only be addressed by a combination of process modifications as well as biocatalyst modifications. Indeed, in many cases it is not clear at an early stage how to develop the process. In order to overcome this, one potential vision for the future could be automated data collection and systematic testing of alternatives at a miniature scale such that operations can be carried out in parallel and with a reduced reagent inventory. This is the main aim of the EC-funded BIOINTENSE project, and the experimental and practical challenges of such an approach have recently been discussed by Krühne and co-workers (2014).[3]

When considering the list of potential process and biocatalyst modifications, analyzing all potential options is a combinatorial problem that is too difficult and time-consuming to be addressed by evaluating options one-by-one in the laboratory, even at miniature scale. However, specifically at this point, mathematical models can be used to supplement biocatalytic process development, and to support the rapid identification of the most promising biocatalytic process options among many. This also matches the above-mentioned ideas on automated data collection and systematic testing of alternatives at a miniature scale. Automated data collection can indeed be combined with automated model structure selection and parameter estimation, as recently illustrated for a conventionally-catalyzed Diels-Alder reaction with complex kinetics in a microreactor.[4]

### Bioprocess development drivers – fermentation

Fermentation processes have been used for hundreds of years in the production of food, including beer and wine. However, partly due to the scarcity of fossil fuels, fermentation processes have become increasingly attractive during the past decades to produce proteins (including enzymes), fine and bulk chemicals as well on the basis of renewable raw materials. The essential difference between a biocatalytic process and a fermentation process is that the catalyst in the fermentation process is a living microorganism – most often a genetically modified organism overexpressing the genes required to produce the product of interest – that grows on a carbon substrate which usually also forms the substrate for the formation of the product of interest. As a consequence, successful implementation of an economically feasible fermentation process relies on achieving a high enough product yield on substrate (especially for lower value products) as well as maintaining a delicate balance between using substrate for biomass growth on the one hand and product formation on the other hand. If biomass growth is not sufficiently prioritized, the product formation rate will be too low, resulting in suboptimal exploitation of the available reactor volume. On the other hand, if biomass growth is promoted too much, the final yield of product on substrate achieved in the fermentation process and the product concentration will be suboptimal. Thus, the main economic drivers of an industrial fermentation process are the yield of product on substrate and the final product concentration that can be achieved – the higher the better, since less water needs to be removed from the product in the downstream processing. Furthermore, for aerobic fermentations the energy cost for oxygen supply is also an important cost.

Mathematical models are often used to study laboratory scale fermentation processes. However, their use in industry is rather limited, and fermentation process development has traditionally relied on an extended series of experiments at lab-scale and pilot-scale in order to find the operating conditions that result in an economically feasible fermentation process. In recent years, microliter and milliliter scale devices capable of performing fermentations have been developed as well,[5] and have been promoted for use in fermentation process development. However, it is quite clear that additional research work is needed before the use of microscale or milliliter scale devices will be the generally accepted process development strategy or support tool. Mechanistic models could, according to us, be helpful in realizing that future vision.

## PSE methods and tools

Process systems engineering (PSE) is an interdisciplinary field within chemical engineering that focuses on the design, operation, control, and optimization of chemical, physical, and biological processes through the aid of systematic computer-based methods. A systems approach is generally model-based, i.e. different types and forms of mathematical models play a prominent role in process design/operation, evaluation and analysis as they have the potential to provide the necessary process understanding, supplement the available knowledge with new data, and reduce time and cost for process-product development.[6,7] PSE methods and tools have been applied successfully to many industries, such as the chemical and petrochemical, the pharmaceutical[8] and biotechnological industries.

While working on a process development task, independent of scale, mathematical models are often used to summarize the available process knowledge and to describe the dynamics of the most important process variables. Such ‘dynamic models’ are usually mechanistic models of a process or a

unit operation, for example consisting of a set of ordinary differential equations (ODEs) which represent the input-output dynamics. Once available, such a model can be supplemented by a set of well-established model analysis tools,[9–11] for example also including uncertainty and sensitivity analysis to assess the statistical quality (reliability) of the simulated scenarios.[12] Perhaps most importantly from a process development point of view, the calibrated dynamic models can be used for *in-silico* testing of a set of potential process operating strategies, e.g. by comparing different control strategies in a series of dynamic simulations, without disturbing process operation. The latter is a major advantage, but requires a dynamic model which has been calibrated on the basis of available process data.

## Case study examples

### Example 1: Bi-enzyme production of lactobionic acid (Santacoloma, 2012)[3]

The main goal of this first example was to analyze the reliability of a mechanistic mathematical model describing a biocatalytic reaction in a lab-scale reactor in terms of its prediction quality. During the process the temperature was controlled at 30 °C and pH was maintained at 3.9. Furthermore, concentrations of lactose, lactobionic acid and oxygen were measured for 6 hours. After that time, the lactose was completely consumed. The sampling interval for lactose and lactobionic acid was 1 hour and the samples were measured by High-performance liquid chromatography (HPLC). The dissolved oxygen measurements were recorded every 10 seconds.

Production of lactobionic acid (4-O-$\beta$-D-galactopyranosyl-D-gluconic acid), a compound used in the production of high-value products, pharmaceutical and food applications, is primarily achieved by the oxidation of lactose. The general scheme for the biocatalytic production of lactobionic acid is shown in Fig. 1. A first enzyme, cellobiose dehydrogenase (CDH), catalyzes the dehydrogenation of lactose to lactobiono-lactone, which is spontaneously hydrolyzed to lactobionic acid. In this case, the double action of the redox mediator 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) is exploited. In the first reaction, ABTS acts as an electron acceptor regenerating the initial oxidation state of the first enzyme (CDH). In the second reaction, ABTS serves as electron donor to obtain the reduction by laccase (lacc), which is the second enzyme added to the system. The reduced state of laccase catalyzes the second reaction where oxygen (the co-substrate) is fully reduced to water.[14,15]

The mathematical model for this system was obtained from the literature, including the kinetic parameters of the multi-enzyme process.[16] and was implemented in MATLAB. Both enzymes involved in the process (CDH and lacc) follow the substituted enzyme mechanism. Kinetic parameters for each enzyme were obtained from the literature.[14,15,17] Interaction due to the combination of enzymes was not taken into account in these studies. In this case study, the bi-enzyme process was carried out in batch mode, in a membrane bioreactor. The main purpose of this reactor was to provide bubble-free oxygenation. Furthermore, the mass transfer of oxygen from the gas to the liquid phase was included in the mathematical model.[16]

The following assumptions were made for the mathematical model: (1) Substrate and product inhibition are neglected in the process; (2) pH and temperature are maintained constant during the operation; (3) Perfect mixing in the reactor.

The model for the system consists of six differential equations, and can be written down in a compact matrix notation,[18] as shown in Table 1. An example of how the matrix in Table 1 should be read is shown in Eq. 1 with the oxygen balance:

$$\frac{\mathrm{d}C_{\mathrm{o}_2}}{\mathrm{d}t} = r_{omt} - \frac{1}{2}r_2 \tag{1}$$

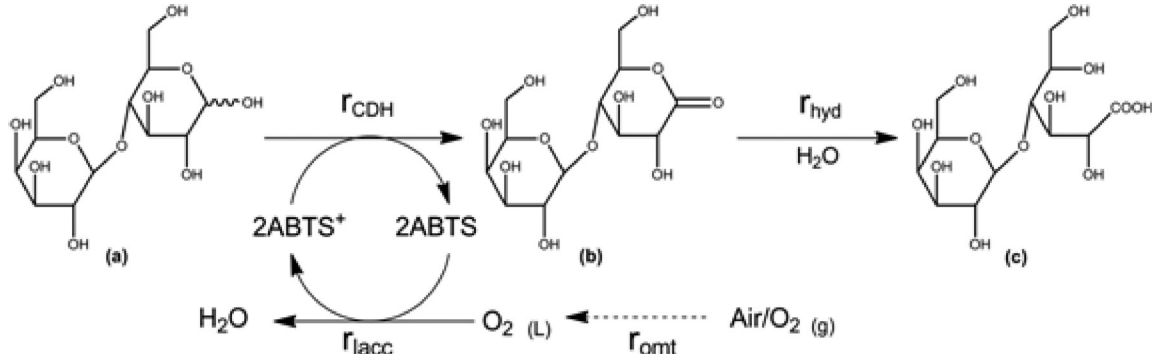


Fig. 1 – *General reaction scheme for bi-enzyme production of lactobionic acid: (a) lactose, (b) lactobiono-lactone and (c) lactobionic acid*

Table 1 – *Mass balances of the batch process for lactobionic acid production represented by the stoichiometric matrix notation*

| Component / Process | $C_{lact}$ (mM) | $C_{LBL}$ (mM) | $C_{LBA}$ (mM) | $C_{O_2}$ (mM) | $C_{ABTS}$ (mM) | $C_{ABTS+}$ (mM) | Process rates |
|---|---|---|---|---|---|---|---|
| Enzyme 1- CDH | –1 | 1 | | | 2 | –2 | $r_{CDH}$ |
| Enzyme 2- Lacc. | | | | –1/2 | –2 | 2 | $r_{lacc}$ |
| Hydrolysis | | –1 | 1 | | | | $r_{hyd}$ |
| Aeration | | | | 1 | | | $r_{omt}$ |

Table 2 – *Reaction rate expressions for lactobionic acid production*

| Reaction rate (symbol) | Reaction rate expression |
|---|---|
| $r_{CDH}$ | $r_{CDH} = V_{max\_1} \frac{C_{Lact} \cdot C_{ABTS+}}{K_{M_{Lact}} \cdot C_{ABTS+} + K_{M_{ABTS+}} \cdot C_{Lact} + C_{Lact} \cdot C_{ABTS+}}$ |
| $r_{lacc}$ | $r_{lacc} = V_{max\_2} \frac{C_{O_2} \cdot C_{ABTS}}{K_{M_{O_2}} \cdot C_{ABTS} + K_{M_{ABTS}} \cdot C_{O_2} + C_{O_2} \cdot C_{ABTS}}$ |
| $r_{hyd}$ | $r_{hyd} = K_{hyd} \cdot C_{LBL}$ |
| $r_{omt}$ | $r_{omt} = K_L a \cdot (C_{O_2}^{sat} - C_{O_2})$ |

The enzymatic reactions follow the bi-bi ping-pong (or substituted-enzyme[19,20]) kinetics. In this case study, both enzymes follow the same type of mechanism. Hence, two coupled substituted-enzyme mechanisms are suggested to describe both enzymatic reactions. The process rates are summarized in Table 2.

Progress curves for lactic acid, dissolved oxygen and lactobionic acid formed the basis of a parameter estimation. Details of the parameter estimation procedure can be found in Santacoloma (2012).[13] The resulting model fit is illustrated in Fig. 2. The parameter estimates, including confidence intervals, are provided in Table 3.

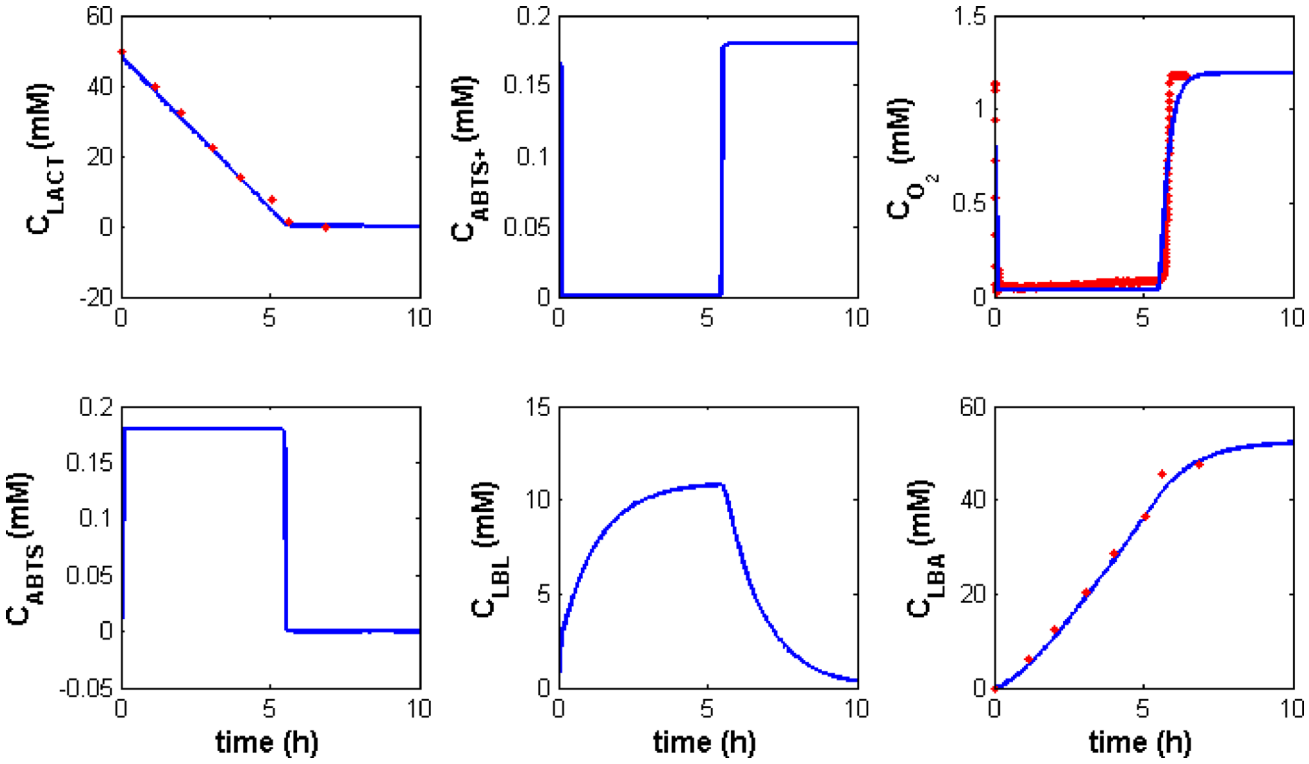


Fig. 2 – *Comparison between experimental data and simulation of the system using the estimated parameters (line – simulation, dots – measurement)*

Table 3 – *Lactobionic acid example: parameter estimates with 95 % confidence intervals and correlation matrix of the estimated parameters*

| Parameter | Estimates with 95 % C. intervals | | Units | Correlation matrix | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | $\theta_1$ | $\theta_2$ | $\theta_3$ | $\theta_4$ | $\theta_5$ | $\theta_6$ | $\theta_7$ |
| $V_{max\ 1}$ | 23.33 | ± 16.4 | mM $h^{-1}$ | 1 | | | | | | |
| $K_{M\ lact}$ | 1.27 | ± 3.06 | mM | –0.47 | 1 | | | | | |
| $K_{M\ ABTS+}$ | 4.10 e–5 | ± 0.09 | mM | 0.85 | –0.71 | 1 | | | | |
| $V_{max\ 2}$ | 58.48 | ± 34.7 | mM $h^{-1}$ | 0.29 | 0.13 | –0.08 | 1 | | | |
| $K_{M\ ABTS}$ | 8.74 e–3 | ± 0.51 | mM | 0.42 | 0.18 | –0.06 | 0.83 | 1 | | |
| $K_La$ | 3.84 | ± 0.10 | $h^{-1}$ | 0.13 | 0.13 | 0.23 | –0.07 | –0.22 | 1 | |
| $K_{hyd}$ | 0.655 | ± 0.44 | mM $h^{-1}$ | –0.00 | 0.00 | –0.00 | –0.00 | –0.00 | 0.00 | 1 |

Despite the assumptions, the suggested mathematical model can in general describe the process dynamics. Seven parameters were found to be identifiable based on the given dataset, but the kinetic parameters ($K_M$) for both oxidation states of the intermediate redox mediator ABTS are very small which physically means fast dynamics in the system as the lactic acid approaches depletion. That effect could probably also explain – at least to some extent – the uncertainty in those parameters, observable in Table 2 as a large confidence interval. Several other parameters show rather large confidence intervals as well. This means[12] that the absolute values of the parameters should be interpreted with care, i.e. the model can describe the process dynamics but the physical meaning of the parameters is limited. Improved quality of the parameter estimation (reduced confidence intervals) could be achieved by collecting measured data on other model variables as well.

### Example 2: CFD to study mass transfer phenomena in microreactors (Bodla *et al.*, 2013)[21]

The second case study demonstrates the combination of microreactor technology and computational fluid dynamics (CFD) to contribute towards understanding of the diffusional properties of substrate and product in a biocatalytic reaction. Such knowledge can then be applied to design new reactor configurations.

As a case study, an ω-transaminase catalyzed transamination for the synthesis of chiral amines was selected. Biocatalytic transamination is studied intensively nowadays, mainly because the transamination reaction is attractive for synthesis of optically pure chiral amines (which are valuable building blocks for pharmaceuticals and precursors). However, in the synthetic direction the reaction is often limited by unfavourable thermodynamics, as well as substrate and product inhibition of the enzyme activity.[22] The reaction is catalysed by ω-transaminase, in the presence of a co-factor, pyridoxal-5'-phosphate (PLP), by transferring the amine group from the amine donor to a pro-chiral acceptor ketone, yielding a chiral amine along with a co-product ketone. The reaction follows the bi-bi ping pong mechanism where the substrate is first bound to the enzyme while co-product is released before the second substrate is bound and the final product leaves the enzyme.[23] Thus diffusion of the substrate to the enzyme binding site and the product diffusion potentially have a significant effect on the reaction performance. Hence, it was specifically intended here to study the diffusion characteristics of the substrate and the product under operating conditions.

Transient experiments were performed in a microchannel under continuous flow conditions. Following a step input of the diffusing species at the inlet at time $t = 0$, the phenomenon of species transport in uniform poiseuille flow is explained by the convection-diffusion equation.[24] A species that is diffusing relatively fast creates a more radial mixing profile, while a species diffusing more slowly has less effect. Under laminar flow conditions, residence time distribution (RTD) experiments were performed by inducing a step input at the inlet of the channel after reaching steady-state, while the concentration over time is subsequently measured at the outlet in order to obtain the response curves, $E(t)$ as shown in Eq. 2. These distribution profiles are helpful in understanding the diffusional properties of each species. Slowly diffusing species have more lag time, and thus it takes more time to reach the normalized concentration at the outlet. The first molecules of the species will also break through sooner at the end of the channel compared to relatively faster diffusing species (Fig. 3).

$$E(t) = \frac{C(t)}{C_o} \tag{2}$$

Where $C_o$ is the species concentration at the inlet for a step input, and $C(t)$ is the concentration measured at the outlet at time $t$. The RTD experiments were performed in the microchannel at a flow rate of 7.5 μL $min^{-1}$ for the amine acceptor substrate (acetophenone), for the amine product (methylbenzylamine), and for glucose, as shown in Fig. 3. The channel dimensions (width $0.5 \cdot 10^{-3}$ m, height $1 \cdot 10^{-3}$ m, length 0.1 m) are sufficiently small and the flow rate is sufficiently low to maintain a laminar flow (Reynolds number is 0.2). Glucose is a compound with a known aqueous diffusion coefficient of $0.67 \cdot 10^{-9}$ $m^2$ $s^{-1}$ and was therefore used as a reference.

Computational fluid dynamics (CFD) models of the flow behaviour were also constructed for a range of diffusion coefficients with the intention of distinguishing between fast and slowly diffusing compounds (i.e. compounds with orders of magnitude differences of their diffusion coefficients). ANSYS CFX version 12.5 was used as software package for this purpose. Response curves were obtained from the simulations, after inducing a step input at the inlet, and by measuring the area average of the species concentration at the outlet of the channel and are also plotted in Fig. 3.

The results in Fig. 3 provide a comparison of the experimental data obtained from transient experiments with the RTD curves resulting from CFD simulations. The simulation result, with a diffusion coefficient of $0.67 \cdot 10^{-9}$ $m^2$ $s^{-1}$, fits well with the data for the product, indicating that the diffusion coefficient of the product is close to that of glucose. With respect to acetophenone, the results indicate an increased lag time to reach the normalized concentration at the outlet compared to the product implying that the substrate is diffusing slower than the product. Compared to the simulations, the experimental data does not fit exactly, although the behaviour of the response curve is closer to that of the simulation with a diffusion coefficient of $0.67 \cdot 10^{-12}$ $m^2$ $s^{-1}$. Hence it can be interpreted that the diffusion coefficient is in the order of magnitude of $10^{-12}$. Thus it can be concluded that the substrate is diffusing considerably slower than the product (around $10^3$ fold slower).

For experimental values, a standard deviation of about 10 % from the mean has been observed. This could account for an error of 10 % in determining the value of the diffusion coefficients. Further errors in numerical simulations will have a combined effect on determining the value of the diffusion coefficients. CFD simulations for solving the Navier -Stokes equations for fluid dynamics are well established in various applications. It is important to replicate the exact geometry including the wall effects and boundary conditions in the simulation since the response curve is a function of these variables. Appropriate meshing of the geometry is also crucial to minimize the numerical error. The finer the mesh size or the higher the number of mesh elements, the more precise will the numerical calculations be. For transient simulations, the time-step is also important when the error has to be minimized. However, there is a tradeoff between the mesh size, the time-step and the required computational time and effort. Thus a compound (such as glucose in this case study) with a known diffusion coefficient can be used to confirm if the simulations are able to predict the experimental data. Assuming about 5 % error in the numerical simulations, the combined error could be in the order of 5 % – 30 %.

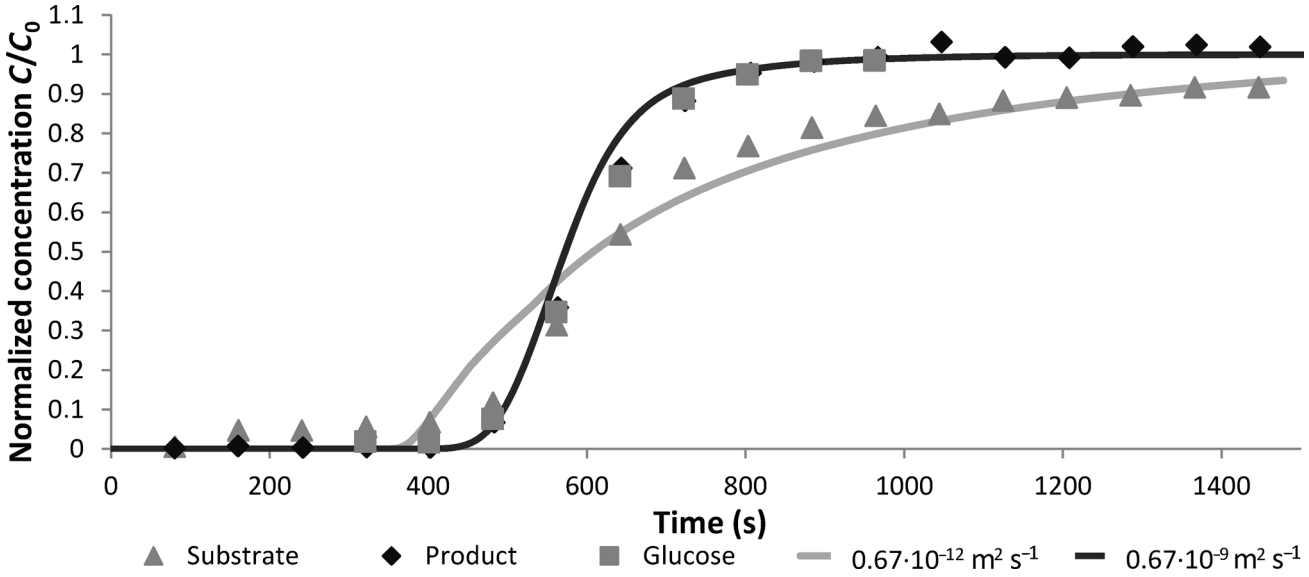


Fig. 3 – *CFD simulations with induced diffusion coefficients of $0.67 \cdot 10^{-9}$ $m^2$ $s^{-1}$ and $0.67 \cdot 10^{-12}$ $m^2$ $s^{-1}$ plotted as continuous lines; Experimental results are plotted as markers. Figure adapted from (Bodla et al., 2013)*[25]

In this case, the substrate is estimated to be diffusing 1000 fold slower compared to the product, where the real value could thus be about 700–1300 times slower compared to the product (assuming maximum 30 % error). So when comparing the numerical response curves with the experimental data, errors in both numerical simulation and experimental data can result in incorrect estimation of the diffusion coefficients.

The knowledge of substrate and product diffusion coefficients is crucial for the choice and design of reactors for biocatalytic reactions. Different reactor configurations can be achieved based on the flow and species transport characteristics. It has been demonstrated that the reactor configurations built from this knowledge perform better than the traditional well mixed batch reactor.[21] In order to build reactor configurations for industrial purposes, it is furthermore also crucial to be able to extrapolate the results from microscale to larger industrial scale. Although it is challenging to obtain the selectivity of a microreactor configuration in a conventional reactor, the data acquired at microscale can be used as a guide to understanding the process limitations during scale-up.

### Example 3: Topology optimization (Schäpper *et al.*, 2011)[25]

The third case study (Schäpper *et al*., 2011),[25] presents a new approach to the design of microbioreactor layouts using topology optimization, a method which had previously been successfully applied in the design of optimal catalytic microreactors.[26] Topology optimization is an iterative mathematical optimization technique which can optimize a design according to the value of a pre-defined objective function. In this case the design was the spatial distribution of immobilized yeast cells and their carrier material inside a small bioreactor, which was optimized based on the yeast cells' total production of a given protein as the objective function.

The yeast *Saccharomyces cerevisiae* was chosen for this study for several reasons: it is one of the best known model systems, and *S. cerevisiae* is furthermore one of the microorganisms most commonly used in the biotechnology industry.

Simulations were carried out using the software COMSOL coupled to MATLAB and the optimized reactor was a rectangular microbioreactor with a length of 1.2 mm and a width of 1.2 mm. A constant pressure difference between inlet and outlet provided a continuous flow of glucose containing medium inside the reactor.

Inside the reactor, the distribution of a carrier material with immobilized yeast cells was then optimized. The carrier was modeled as a porous, sponge-like material which gave rise to an additional so called Darcy friction anti-parallel to the flow medium. For the volumes inside the reactor with no carrier present, i.e. those regions only containing culture medium, the Darcy friction was set to zero.

For a given distribution of carrier material in the reactor, the flow velocities of the medium were calculated from the steady state Navier-Stokes equation, taking the Darcy friction of the carrier material into consideration. These flow velocities were then used in the second part of the calculations, where kinetic models were applied to model the protein production in the reactor.

Topology optimization was then applied in order to find a better reactor design with a more beneficial distribution of carrier material, and each candidate was evaluated based on how high a protein production the configuration could achieve.

The kinetic model in this study was based on the work of Bránayik *et al*. (2004)[27] and Zhang *et al*. (1997),[28] and describes the yeast metabolism through the three metabolic events described in Fig. 4.

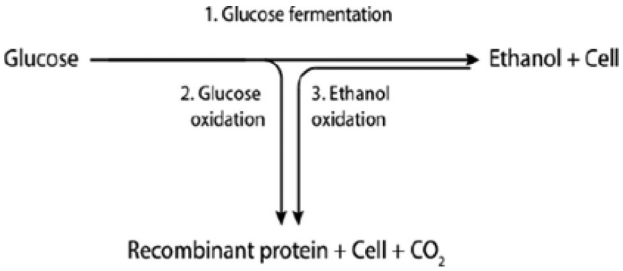


Fig. 4 – *The three pathway model for yeast metabolism suggested by Zhang et al. (1997).[28] (Figure adapted from (Schäpper et al., 2011)[25])*

According to the model, glucose may be oxidized to carbon dioxide along the respiratory metabolic pathway 2. However, if the glucose flow becomes too large for the respiratory capacity of the cell, excess glucose is fermented to ethanol according to pathway 1, and the activity of the enzymes in the glucose oxidation pathway is reduced. When glucose approaches depletion, ethanol begins to be metabolized by pathway 3. The cells grow exclusively on ethanol when glucose is exhausted.

In this model, the production of the desired protein is assumed to be associated with growth and is exclusively associated to the oxidative metabolism (pathways 2 and 3) in the yeast cells. This means that the production of the protein will be negatively affected by, for example, too high glucose concentrations.

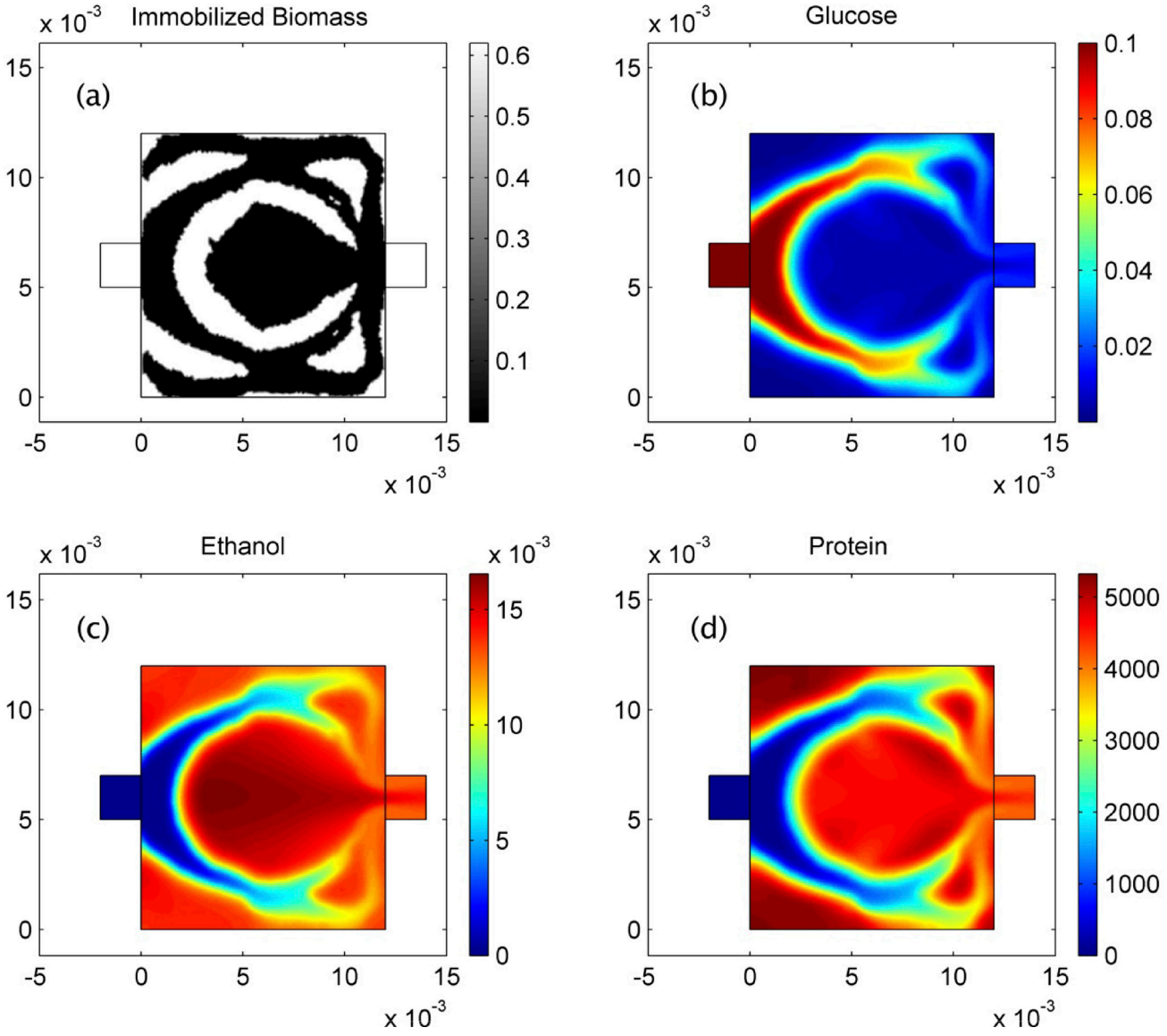


Fig. 5 – *Resulting structure and concentrations for a glucose inflow concentration of 0.1 g L$^{-1}$. (a) Distribution of biomass where white = cells and black = fluid, (b) glucose concentration [g L$^{-1}$], (c) ethanol concentration [g L$^{-1}$] and (d) protein concentration [units L$^{-1}$]. From Schäpper et al. (2011).*[26]

With this as a basis, a set of equations describing glucose consumption, ethanol production and consumption, protein production as well as both immobilized and suspended biomass was implemented as a kinetic model. The concentrations of glucose, ethanol, protein and biomass were then calculated at steady state based on the kinetic models coupled to their diffusion in the medium as well as their convection, based on the previously calculated flow velocities. From this the objective function, which was the total production of protein in the system, was calculated and the carrier distribution re-organized in order to try to find a more optimal distribution, by repeating the flow and kinetic calculations.

The total protein production in the optimized bioreactors (i.e. in the reactors with an optimized distribution of carrier) was then compared to the calculated performance of non-optimized reactors (i.e. in reactors where the carrier material was homogeneously distributed).

This comparison was made for different glucose concentrations in the feed and the results can be seen in Table 4, which shows that the protein mass flow rate at the outlet increased at least fivefold for all the simulated glucose concentrations when topology optimization was applied. The resulting structure for the case with a glucose concentration of 0.1 g L$^{-1}$ in the feed can be seen in Fig. 5, together with its resulting glucose, ethanol and protein concentrations at steady state.

The significant gain in protein concentration can be explained by the fact that a structurally optimized distribution, where flow is distributed and islands of biomass are surrounded by streams of liq-

Table 4 – *Comparison of the total protein outputs for the homogeneous and the optimized reactor at different glucose feed concentrations*

| Glucose feed conc. (mg $L^{-1}$) | Protein flow at the reactor outlet (U $sec^{-1}$) | | |
|---|---|---|---|
| | homogeneous reactor | structurally optimized reactor | increase (fold) |
| 1 | 0.3 | 2.7 | 5.8 |
| 5 | 1.4 | 12.9 | 9.1 |
| 10 | 2.7 | 23.1 | 8.4 |
| 30 | 7.2 | 57.4 | 8.0 |
| 50 | 10.7 | 91.7 | 8.5 |
| 100 | 17.6 | 170.3 | 9.7 |
| 200 | 25.2 | 229.5 | 9.1 |
| 500 | 39.0 | 325.2 | 8.3 |
| 1000 | 63.8 | 380.4 | 6.0 |

uid flow, allows for a more balanced distribution of glucose across the reactor leading to higher local protein production rates.

This first theoretical investigation of the potential of topology optimization for improvement of microbial cultivation processes at micro scale has clearly shown that the use of this methodology can potentially lead to microbioreactors with a significantly higher productivity than conventional reactor designs where immobilized biomass is homogeneously distributed.

## Discussion

The presented case studies have different levels of complexity, and address different experimental scales as well. For the first case a lab-scale biocatalytic reaction is described by a system of coupled algebraic and ordinary differential equations that have been solved for a number of state variables, while for the second case, a microreactor, the Navier-Stokes equation has been solved with a mass balance for two different slow diffusing species. Finally in the last case study the partial differential equation systems for momentum and mass transport have been coupled with the kinetic rate laws of a relatively simple biological model, and this model of a microbioreactor was then linked with an optimization routine.

In the case studies, different types of information can be gathered from the calculations. In the first example, a model is confirmed with respect to the prediction quality, which by calibration may be further improved. In the second example a CFD model is applied in order to gain a better understanding of existing experimental data collected in a microscale reactor. Here new insight is quickly gained from a rapidly performed experiment, and this new information – the diffusion coefficient – can subsequently be used for the prediction of later experiments. Finally, the third example is completely theoretical and describes how an advanced model is used with the intention of generating new design configurations of an otherwise relatively well known fermentation system. The future challenge here is to verify experimentally whether new and intensified reaction systems can be generated. An evolutionary algorithm is furthermore implemented in order to achieve this goal.

Such examples are interesting from a scientific point of view, but also the more practical oriented scientist or engineer should consider the more systematic use of PSE methods and tools, since these methods and tools offer a range of convincing opportunities, as well as saving considerable resources. Indeed guiding experimentalists to the most valuable experiments is a key role of PSE methods and tools in general, and modeling in particular.

In most cases it is impossible to investigate all potential process configurations experimentally. Indeed, there is often not enough material (substrate, enzymes and other reactants) available, and if so the time/manpower for the experiments is limited. PSE methods can assist here as well. A broad range of theoretical configurations can be tested in relatively simple simulations and hence the impact of product inhibition, substrate inhibition, co-factor inhibitions and especially also mass transfer limitations due to reactor designs can be tested. A sensitivity analysis[12] is helpful for planning of experiments which can be used for the Design of Experiments (DoE) or Optimal Experimental Design (OED). The sensitivity analysis – local or global – will for example give an indication of which variables to measure in order to allow estimation of specific parameters. New process options can be investigated as well, before they are experimentally tested. In this way, PSE methods and tools can support process development. Even more importantly, PSE methods and tools can support process development in a structured way, meaning that the tools can be used over and over again each time a new process development task is started up.

Another area of application is the direct coupling of experimental data and mathematical simulations. Here well-established models will help to access requested but not available information. For example in case study 2 the diffusion characteristics of acetophenone and methylbenzylamine were not known and could not be found in literature. A surprising result was that by an appropriate experimental design (again planned with help of a model) it was discovered that one of the species diffuses sub-

stantially slower than the other. This was unexpected, since the molecular weight and the chemical structure are very similar. The acquired material properties are fundamentally important for the mass transfer limitations in the reaction and hence this information can also be used for scale up and scale out of reactors and processes.

From an intellectual point of view most interesting is the application of models for testing of concepts and even generation of entirely new ideas. It is not important, that the model predicts correctly from a quantitative point of view. As long as the qualitative prediction capacity is sufficient, the models can be used for the generation of understanding, insight and evaluation of new ideas. The user can visit the virtual laboratory in order to test simple relationships, complex interactions between different kinetic formulations and material transport limitations or simply to obtain a different view of a problem which the user is assumed to have been working with already for a long time. The more exact and experimentally validated the models are, the user might even omit the experimental validation of the simulation. This is classically done in engineering areas like turbine design or ship design, where the fabrication of prototypes is too demanding with respect to the costs.

The impact of the PSE tools can be substantial when the interdisciplinary nature of the project is guaranteed by a proper collaboration of different experts, such as protein scientists, chemists, process engineers, mathematicians and physicists. Then today futuristic appearing models can be used for advanced optimization routines, where under the assumption that the model is right, complex configurations can be automatically produced and hence reactors can be optimized with respect to topology and shape.

A last important potential application area for PSE methods is the transfer of experimentally established knowledge across scales. Miniaturized reactor technology is receiving increased attention due to the economic potential with respect to reduced time and costs in process development. But even though more and more companies are using or experimenting with such technology it is still unknown to what extent the experimental results can be used for the comparison with setups at another scale.

As presented in Table 5, the experimental setup of micro-scale experiments is dominated by laminar flow conditions and hence the mixing is poor and often diffusion limited. This results in considerable material transfer limitations and hence partial differential equations (PDE) have to be solved, for instance by use of CFD models, in order to predict the conditions in such systems. When changing to bench or pilot scale experiments it can be assumed that the systems are relatively well mixed and the mathematical description can be reduced to ordinary differential equations (ODEs), which simplifies the mathematical description of those systems. At full scale the situation is again such that there are mixing limitations due to the physical reactor design and a limited transfer of kinetic energy in comparison to bench/pilot scale setups. The fluid dynamic conditions are here highly turbulent and hence more complex PDE systems (CFD models) have to be applied which also consider turbulence modeling. Under the assumption that

Table 5 – *Summary of the variation of reactor characteristics and model tools across reactor scales*

| Scale | Characteristics | Models |
|---|---|---|
| Micro-scale | Not well mixed, laminar flow, material transport limitations | PDEs (CFD) |
| Lab scale | Well-mixed | ODEs |
| Pilot scale | Usually well-mixed | ODEs |
| Full scale | Often not well mixed, gradients | PDEs (CFD), ODEs (compartment model) |

1. The kinetics can be transferred across scales and
2. The model analysis tools can be used at all scales

it will be possible to answer many open questions with respect to the varying performances of systems at different scales, which is a research area in biochemical process technology which receives considerable attention nowadays.

According to the complexity of the presented case studies also the requested mathematical skills, knowledge and experience of the user has to be appropriately matching the task. For the first case study an experienced student, working for instance on a master project, might be the appropriate person to perform the task. As here presented, the system is modelled with help of MATLAB and mass balances which are coupled with the governing kinetic reaction rate expressions. In the second case study a commercial CFD software (ANSYS CFX 12.5) has been used, which made the numerical investigation simple with respect to the CFD work (days). But it should be considered that a commercial license of such software might not be available at all companies or research institutions. This would then demand either an investment into a license or the use of open software, where the latter then would need considerable training for the person involved. Finally in the third case, again a commercial CFD software (COMSOL) has been used and coupled with an evolutionary algorithm written in MATLAB. Clearly this is the most advanced PSE example that is presented here and a considerable experience

with this software tool has been a requirement. Consequently, the user of this software has been an advanced user and has nevertheless spent a considerable amount of time (month) on this task.

## Conclusions and perspectives

This article has briefly presented an overview about how Process System Engineering (PSE) methods can be used for the systematic development of (bio) reactor systems. Three case studies have been presented with different applications, reactions and scales. The intention of the studies is to present different applications of PSE tools. One important focus area is the use of PSE methods for the development of miniaturized reactor systems. It was demonstrated, how models can assist in achieving a better understanding of the process conditions, the prediction of process performance and the theoretical investigation of reaction conditions with computer based algorithms for reactor improvement. The manuscript gives the reader a motivation for the use of PSE models and tools at different scales and level of detail of applications. This included practical aspects like determination of material constants or reaction performance as well as more academic use like in optimization routines. The future and experimental studies will show if such *in silico* investigations will contribute to the reduction of process development costs and improved understanding of processes across scales.

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### List of symbols and nomenclature

#### Abbreviations

CDH – Cellobiose dehydrogenase
ABTS – 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt
$ABTS^+$ – 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt cation radical
HPLC – High-performance liquid chromatography

#### Nomenclature

$V_{max}$ – Maximum initial velocity of an enzyme, mM $h^{-1}$
$K_M$ – Michaelis-Menten constant, mM
$K_La$ – Volumetric mass transfer coefficient, $h^{-1}$
$K_{hyd}$ – Hydrolysis constant, $h^{-1}$
$C_0$ – Initial concentration of any species, mM
$C$ – Concentration of any species, mM
$r$ – Reaction rate, mM $h^{-1}$

#### Subscripts

lact – Lactose
LBL – Lactobiono-lactone
LBA – Lactobionic acid
$O_2$ – Oxygen
ABTS – Reduced redox intermediate
$ABTS^+$ – Oxidized redox intermediate
omt – Oxygen mass transfer

#### Superscripts

CDH – Cellobiose dehydrogenase
lacc –Laccase
$ABTS^+$ – Oxidized redox mediator
ABTS – Reduced redox mediator
sat – Saturation

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