Project Description – Project Proposals

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Adaptive Optimal Control of Continuous Aqueous Two-Phase Flotation (ATPF)

Project Description

1 Starting point

1.1 State of the art and preliminary work

The global challenges of today are very dynamic. However, to meet the challenges, we can rely on new technologies. In this context, biotechnological products play a key role in finding solutions. Within a year, novel mRNA vaccines have been approved in the fight against the Corona pandemic. In mRNA vaccines, the active ingredients are encapsulated within liposomes or liquid-nano-particles (LNP). Thereby, phospholipids form an outer shell around the active ingredient and ensure the transport in the human body. Due to their high bioavailability, phospholipids are also used for various formulations in oral drug delivery or as active ingredients themselves [11, 12].
Specific modifications of the amphiphilic phospholipids by enzymes, for example the cleavage of fatty acid from the hydrophobic tail allow one to stabilize different active ingredients [13, 14]. The enzymatic modifications can change the gel phase transition temperature, and the wetting characteristics of the phospholipids. There are various phospholipases, each of which hydrolyzes phospholipids at different sites and thus specifically alters their properties for an improved quality [15]. Phospholipases are technical enzymes that play a crucial role as biocatalysts in many industrial applications. Their unique properties are used not only in the formulation of pharmaceutics but also in cosmetics and food production [14]. This leads to a large demand for phospholipases, which can only be met by microbial fermentation. In large bioreactors, microorganisms express the enzymes and discharge them from their cells into the surrounding fermentation medium. There, they are usually present in low concentrations in an aqueous environment and must first be purified and concentrated, which can be realized by aqueous two-phase flotation (ATPF). This alternative downstream process features high process intensity and overcomes the following challenges and disadvantages of traditional enzyme purification:

- The complexity of biosuspensions is high. For a purified product, the phospholipases must be isolated from the other particulate and dissolved components in the fermentation broth. Starting with cell separation by microfiltration (drum filters) or centrifugation (disc separators) leads to a high energy demand, caused by pumping or accelerating the biosuspension. Further purification faces the recovery of the phospholipases from the now cell-free biosuspension including the isolation from impurities such as other proteins present in the fermentation broth and substrate residues . Multiple separation steps are needed and hence the equipment effort, energy costs and product losses add up and lead to a main cost share of the downstream in enzyme production.
- The concentration of the enzymes in the aqueous biosuspension is low. Concentrating the enyzmes by ultrafiltration lead to a further high energy input and product loss. Consequently, the effort required to concentrate the biomolecules decreases the energy efficiency and product yield of the manufacturing process even further.
- The activity of the phospholipases (the amount of phospholipids that can be hydrolyzed per time) is decisive for the quality of the enzyme product. Since enzymes are sensitive macromolecules with spatial orientation, large number of separation and purification steps with sometimes long residence times and harsh conditions lead to a drastic loss of enzyme activity.

- Technical enzymes are often produced in continuous fermentation processes. This reduces the required size of the bioreactors and prevents product limitations or substrate inhibitions and guarantees an improved space-time yield. Continuous phospholipase upstream presupposes a likewise continuous downstream in order to avoid intermediate storage and to achieve the lowest possible retention times. At the same time, the purification process must be robust and controllable in order to be able to react to fluctuating phospholipase concentrations due to the dynamic upstream.

For high resource and energy efficiency, an integrative downstream process is needed to meet the challenges of enzyme purification. The purification of phospholipases by ATPF can save numerous separation and purification steps and yield a highly concentrated enzyme solution with high purity and phospholipase activity. Continuous purification by ATPF significantly increases the time-space yield compared to batch operation [2]. Online monitoring and a suitable control strategy are expected to further improve the efficiency of the separation process. An autonomous process control is necessary because the ATPF input properties in a production chain are often subject to fluctuations. Product requirements imposed by the operator may also change as well as external disturbances. When sensors detect the resulting discrepancies, the most efficient way to counteract is immediate, autonomous compensation by the controller. However, the controller needs to be adapting to the process in order to take sensible measures because the stability of the two-phase system must be ensured permanently and not be disrupted by overshooting control action.
The aim of this project is therefore to convert continuous ATPF into an autonomously controlled process for phospholipase recovery and purification that can be easily transferred to other biotechnological products from complex biosuspensions. The experimental focus is being handled by the Institute of Mechanical Process Engineering at the Karlsruhe Institute of Technology (Prof. Hermann Nirschl), including the establishment of suitable online measurement methods to determine the concentration and activity of the phospholipases. In close cooperation with the Institute of Microsystems Engineering of the University of Freiburg (Prof. Moritz Diehl) a new system model of the continuous ATPF process is created. The system model enables two-stage nonlinear model predictive control (NMPC), which is developed in Freiburg and combined with an estimation method capable of learning zero- and first-order model corrections online to ensure optimal steady-state operation under various process conditions. In addition, parametric model uncertainties as well as external perturbations are explicitly taken into account with uncertainty aware optimal control problem formulations.
At the end of the first funding period, a closed-loop control system, that is able to adapt the ATPF model and to react to disturbances based on online measurements, shall be available on a laboratory scale. In the second funding period, the further separation of phospholipases from the concentrated enzyme solution by ultracentrifugation will be integrated into the controlled process chain. The following sections describe in more detail the principles of ATPF, an approach to model building and adaptive optimal control for continuous ATPF, including the state of the art, previous work, objectives and work program of the research project.

Aqueous Two-Phase Flotation (ATPF)

State of the Art. ATPF is a combination of aqueous two-phase extraction (ATPE) and flotation. It was first described 2009 by Bi et al. [16] for the separation and concentration of penicillin G from fermentation broth. For both, ATPF and ATPE an aqueous two-phase system (ATPS) forms the basis of the process. By mixing phase-forming components of two different species (mostly polymers and salts) in sufficiently high concentrations, an aqueous solution is split into a top phase and a bottom phase. Water forms the main component of both phases, which is the reason for the high biocompatibility of ATPS. In polymer-salt systems, the top phase usually has a high polymer concentration and a low salt concentration, while the opposite is true for the heavier bottom phase. For the purpose of extracting biomolecules, the composition of the two-phase system is chosen

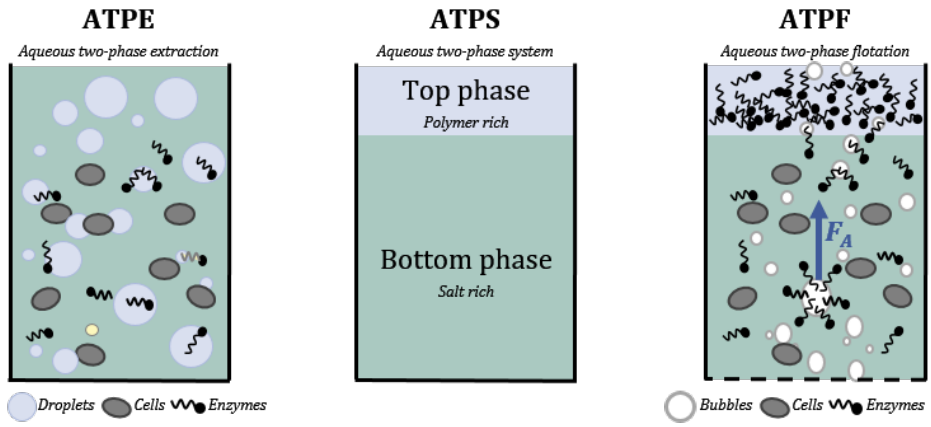

Figure 1: Schematic illustration oft the basic principles of aqueous two-phase systems (ATPS), aqueous two-phase extraction (ATPE) and aqueous two-phase flotation (ATPF)

such that the product shows an increased affinity for the top phase. In the ATPE, the top phase is dispersed as small droplets in the bottom phase and the biomolecules diffuse into the top phase droplets. Without input of mixing energy, the two aqueous phases separate due to their density difference and the biomolecules are thus concentrated in the top phase, whereas impurities (e.g. microorganisms) maintain in the bottom phase. In ATPE, a large volume of top phase must be dispersed in the product-containing bottom phase, since this creates the necessary exchange surface for diffusive mass transfer. in ATPF, the top phase is not dispersed in the bottom phase, but gas bubble introduction takes place at the bottom of a flotation cell. The biomolecules attach themselves with their hydrophobic regions to the bubble surface, rise with the gas bubbles and are thus transported into the collecting top phase, where they remain after the gas bubbles burst. Since the main mass transfer is realized by flotation, no large amounts of top phase are need to be dispersed for rapid mass transfer. This results in low costs for phase-forming components and in high concentration factors of the product in the liquid top phase. The principles of ATPS, ATPE and ATPF are illustrated in Fig. 1.

ATPF combines the material advantages of aqueous two-phase systems with the high mass transfer of flotation. This allows biomolecules to be selectively separated from complex biosuspensions and concentrated in a collecting top phase. Both pharmaceutical and technical molecules [16–20] and enzymes [1, 2, 21–23] can be separated from plant extracts or fermentations with mircoalgae, bacteria or fungi. Lee et al. [24] summarized in a review the research on ATPF and justified the great potential in direct recovery of biotechnological products by ATPF in four main advantages: High separation efficiency, high concentration factors, environmental friendliness, and cost effectiveness. The last two points result from the reduced use of top phase and, consequently, the low requirement for phase-forming polymer compared to ATPE. While in ATPE the volume ratio of top phase to bottom phase ($V_{\mathrm{TOP}}/V_{\mathrm{BOT}}$) is usually in the range of 1:2 - 1:5, ratios of 1:4 - 1:30 are common in ATPF [24].

Although ATPF has been well investigated on the material side (product, ATPS, pH), almost no process optimization has been carried out to date. Only recently the important influence of the gas input could be shown [1]. Furthermore, a continuous ATPF was presented for the first time, which leads to a significant increase in space-time yield [2]. These findings allow one to optimize ATPF by process engineering to design an industrially applicable process. The latter results are explained in more detail in the following section.

For an efficient continuous separation, process monitoring and control is essential. For this purpose, the input and output variables of the separation step must be measured online. In the case of enzyme purification by continuous ATPF, the enzyme concentration and the enzyme activity at the

inlet and outlet of the apparatus are decisive variables. The enzyme concentration can be measured by simple light absorption measurements in the range of 280nm due to the aromatic amino acids absorbing light of this wavelength. The enzyme activity describes the catalytic property of the enzymes, i.e. how much substrate can be converted per time, and is thus a measure of the quality of an enzyme product. To measure phospholipase activity, substrate conversion or product formation must be recorded. A fast and simple principle to monitor phospholipase activity is based on a colorimetic enzyme assay. During the phospholipase reaction, fatty acids are cleaved from phospholipids, which releases oxonium ions. By adding a pH-sensitive indicator, the acidification can be tracked and thus inference can be made about substrate turnover [25, 26]. Even though the application of colorimetric activity assays for phospholipases is widespread, there are no reports of a colorimetric online assay yet, which is necessary for monitoring a continuous process.

Preliminary Studies. The Institute for Mechanical Process Engineering and Mechanics at KIT is concerned with the purification of valuable products from complex suspensions. Various processes such as centrifugation, filtration and flotation are optimized and linked in process chains. Also, aqueous two-phase flotation is intensively investigated. It could be shown, that the enzyme phospholipase A2 can be separated from a biosuspension by ATPF. Here, the gas input is crucial for the efficiency of the separation [1] and continuous operation of the process significantly increases the space-time yield [2]. The core results of this work and other preliminary experiments are summarized below.

Gas input into the bottom phase can occur via different porous media in ATPF, resulting in different bubble size distributions. The bubble size is crucial for both, enzyme transport and the stability of the aqueous phase boundary between top and bottom phase. In general, the smaller the gas bubbles and the higher the gas volume flow rate, the higher the interfacial entry and thus the total bubble surface area to which the enzymes can adsorb. Even if the target is a high gas bubble entry, the gas flow rate cannot be chosen arbitrarily high, since this also entails an increase in bubble size. Bubbles that are too large rise very quickly and swirl in the bottom phase as they pass the top phase boundary. Bubbles that are too small, on the other hand, do not manage to overcome the interfacial tension and get stuck at the phase boundary. For the selected two-phase system of PEG 1000 and citrate, the ideal bubble size window is therefore in the range between 281 µm and 543 µm [1]. The gas input studies in the ATPF allow an estimate to be made of both, how much phospholipase can be transported into the top phase per time and the risk of phase instability, depending on the selected gassing medium and the set gas flow rate.

The phase turbulence can additionally also be measured. In the bottom phase, there is a high conductivity due to the high salt concentration. In the polymer-rich top phase, the conductivity is lower due to charge shielding by the polymer molecules. If the top phase is dispersed in the bottom phase due to excessive turbulence input, the conductivity decreases. Thus, by measuring the conductivity in the bottom phase, the unwanted phase swirling can be detected and quantified.

In addition to the stability of the process, the quality of the enzyme product, i.e. the enzyme activity, is crucial. By a colorimetric assay, the hydrolytic cleavage of the phospholipid lecithin can be observed and thus the phospholipase activity can be determined. Such an assay has previously been successfully applied offline by sampling during batch ATPF. In Fig. 2, the time course of separation efficiency for batch ATPF of a phospholipase is shown. After an initially steep increase, the increase and thus the transport of phospholipase molecules to the top phase flattens out after approximately 40 min of flotation time. A similar course can be observed for the phospholipase activity, where there is even a slight decrease in the further course of flotation after 40 min.

It is found that most of the mass transfer occurs at the beginning of batch ATPF, when the phospholipase concentration gradient between enriched bottom phase and unloaded top phase is highest. To take advantage of this effect, continuous phase exchange was implemented. After 30 min of flotation time, both phases were continuously exchanged by pumping. The pumping rates were selected so that the residence time of the enzymes in the flotation cell was sufficiently high to

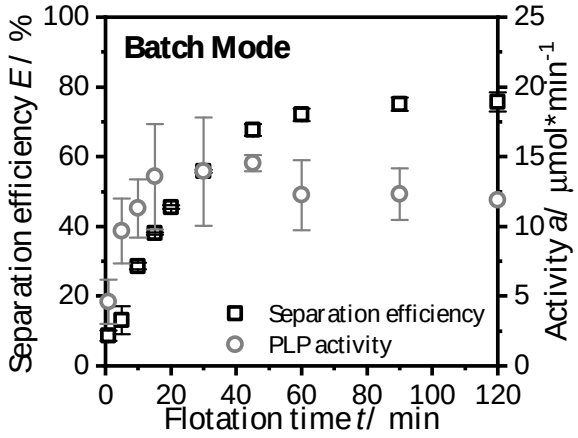

Figure 2: Separation efficiency and phospholipase activity during batch ATPF

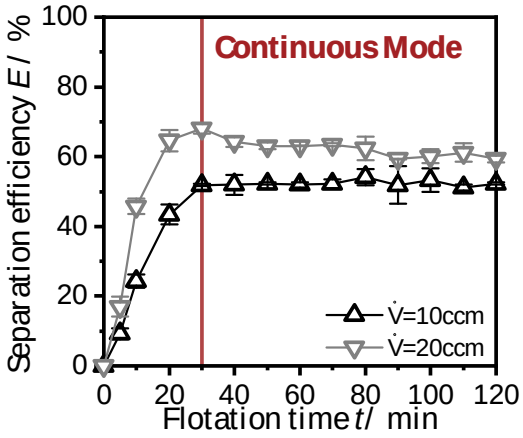

Figure 3: Separation efficiency for two gas flow rates during continuous ATPF [2]

floate them into the top phase. In Fig. 3, the separation efficiencies for two continuous ATPFs with different gas flow rates are shown and a constant curve in continuous mode (between 30 and 120 min flotation time) is evident. The continuous mode made it possible to process 2.5 times the volume of phospholipase-loaded bottom phase in the same time compared to the batch mode. [2]
For continuous ATPF, a horizontal flotation tank in which the phases move in cocurrent is advantageous. As a result, there is a maximum concentration gradient between top or bottom phase at the inlet of the tank, which favors the transfer of phospholipase to the top phase. It also allows multiple gassing units to be integrated along the length, allowing for a higher gas bubble input and thus a higher phase throughput. Such a tank was designed (illustrated in Fig. 4) on a laboratory scale and fabricated using 3D printing. In the design of this continuous ATPF apparatus, attention was paid to maximizing flow control and scale-up capability, so that the tank could be easily manufactured at pilot scale and transferred to an industrial scale.

Modelling of the Continuous ATPF Process

State of the Art. Besides monitoring, modeling mass transfer during ATPF is crucial to set operational parameters such as flow rates and gas volume flow in a model-predictive setup. So far, complete model descriptions are lacking, but there are several known relationships between input, output and process parameters [1, 2]. In this context, it is conceivable to couple modeling approaches of other separation processes with the findings on ATPF. In model-based process control, the focus lies on keeping model complexity low and thus calculation times fast. The fundamental approach is applying the conservation laws for mass, volume, number and energy on the process chamber. This leads to a set of balance equations and applies to steady-state as well as dynamic modeling. Model accuracy can be enhanced incorporating further knowledge on physical effects by simply extending the set of equations. For example, Oosthuizen et al. set up a dynamic model for a chain of flotation cells in which they balance every cell regarding volume and mass [27]. They additionally incorporate prior process and phenomenological information.
Since spatial dependencies often matter for the solution of the balance equations, higher spatial resolution is required. A useful approach is to divide the chamber in compartments, set up balance equations likewise and then interlink the compartments via the balanced streams. Such a compartment approach has been applied to solid-bowl centrifuges and is further explained in the following paragraph.

Preliminary Studies. The Institute for Mechanical Process Engineering and Mechanics at KIT has long-standing experience in process modeling on different scales, complexities and with different methods. In recent years a real-time compartment models for centrifuges have been developed

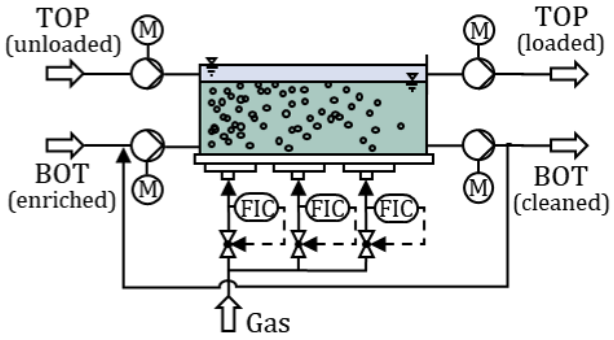

Figure 4: Schematic illustration of ATPF tank.

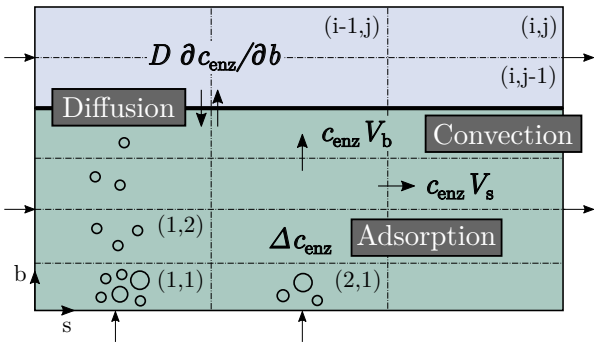

Figure 5: Model scheme.

and proved successful firstly in describing the process itself [3, 28]. Herein, material-specific behavior is covered by means of short-cut equations. Preliminary studies have shown that the material parameters included can be tuned to actual process data by means of numerical optimization, if inaccessible via isolated experiments. Applicability to scale-up from laboratory to pilot- and even industrial scale was demonstrated for decanter centrifuges [29], as well as the embedding of the compartment model together with a neural network in a grey-box model set-up, paving the way for future utility of the model in manifold directions. Additionally, the compartment approach has been successfully implemented in model-based control of a tubular centrifuge to achieve desired centrate properties [5]. Studies have shown that the model is applicable to model-predictive control in various applications and it is thus conceivable to apply the same approach on ATPF.

In preliminary studies towards this proposal, a compartment model for the ATPF process was conceptualized and is shown in Fig. 5. Firstly, the ATPF basin is spatially discretized into compartments (i, j) along the direction of solution (s, counter i) and bubble flow (b, counter j). In s-direction the discretization may be defined by the amount of gas inlets, while the number of compartments in b-direction defines the achievable level of detail. The ATPF basin is then represented by correspondingly dimensioned matrices for enzyme concentration $c_{\mathrm{enz}}(i, j, t)$, bubble concentration $c_{\mathrm{b}}(i, j, t)$, bubble size distribution $p_{\mathrm{b}}(d, i, j, t)$ and size dependent enzyme loading of the bubbles in the compartments $l_{\mathrm{b}}(d, i, j, t)$, where d represents the bubble diameter. Secondly, the boundary conditions are defined, e.g., the current inflow and enzyme concentration of solution at $i = 1$ and the inflow and size distribution of bubbles at $j = 1$. Goal of the proposed compartment approach is to facilitate the formal description of the ATPF process in terms of semi-explicit differential algebraic equations (DAE) of index 1, i.e., the dynamics can be written in the form

$$\dot{x}_{\mathrm{d}} = f_{\mathrm{d}}(x, u), \quad 0 = f_{\mathrm{a}}(x, u), \tag{1}$$

with states $x = (x_{\mathrm{d}}, x_{\mathrm{a}}) \in \mathbb{R}^{n_x}$, controls $u \in \mathbb{R}^{n_u}$ and $\frac{\partial f_{\mathrm{a}}}{\partial x_{\mathrm{a}}}$ invertible. The differential states x_{d} comprise the local enzyme concentrations, as well as possibly bubble concentration and size distribution. As an example, the analytical form for the enzyme concentration considers all contributions visualized in Fig. 5 and may have the form

$$\begin{aligned}
\frac{\partial c_{\mathrm{enz}}}{\partial t}(i, j, t) = \quad & V_{\mathrm{s}} \left[c_{\mathrm{enz}}(i-1, j, t) - c_{\mathrm{enz}}(i, j, t)\right] + V_{\mathrm{b}} \left[c_{\mathrm{enz}}(i, j-1, t) - c_{\mathrm{enz}}(i, j, t)\right] && \text{(2a)} \\
& - \int K_{\mathrm{a}}(d) f \left(c_{\mathrm{enz}}(i, j, t) c_{\mathrm{b}}(i, j, t) p_{\mathrm{b}}(d, i, j, t)(1 - l_{\mathrm{b}}(d, i, j, t)\right) \mathrm{d}d && \text{(2b)} \\
& + D(i, j+1, j) \left[c_{\mathrm{enz}}(i, j+1, t) - c_{\mathrm{enz}}(i, j, t)\right] + \ldots && \text{(2c)}
\end{aligned}$$

Incorporated herein are the contributions of convective transport of free enzyme (2a) and bubbles, adsorption of enzyme onto the bubbles (2b) and diffusion at the liquid-liquid interface (2c). The algebraic states x_{a} might include known relationships, e.g., on the effects of the gas flow rate.

Adaptive Control for Uncertain Nonlinear Processes

State of the Art. For dynamic processes where only approximate models are available, control strategies that adapt the model online need to be employed. In addition to systematic model errors and parametric uncertainties, external perturbations are to be expected. Uncertainty-aware optimal control problem formulations can improve the control performance significantly and ensure constraint satisfaction in the presence of these uncertainties.
To compensate for unknown model dynamics, a promising approach is the so-called modifier adaptation (MA) method for real-time optimization [30, 31]. Within this framework, plant optimality, i.e., optimality with respect to the unknown process dynamics, is achieved by iteratively modifying both the cost and the constraints of a nominal optimal control problem. An excellent overview of MA methods is given in [32]. An alternative MA method, first introduced in [31], adapts instead of cost and constraints the model equation. We plan to follow this second approach and combine the MA idea with an underlying Nonlinear Model Predictive Control (NMPC) scheme similarly to the control strategies proposed in [33, 34]. In particular, the steady-state economic optimization problem using a corrected model takes the form:

$$(u^{\mathrm{ss}}, x^{\mathrm{ss}}, \hat{y}^{\mathrm{ss}}) = \arg\min_{u,x,y} \; H_0(u,y) \quad \text{s.t.} \quad \begin{cases} 0 &= f(x,u;\theta), \\ y &= g(x,u;\theta), \\ 0 &\geq H_i(u,y), i = 1,\dots,n_H, \\ 0 &\geq G(u), \end{cases} \tag{3}$$

where $H_i : \mathbb{R}^{n_u} \times \mathbb{R}^{n_y} \to \mathbb{R}$ and $G : \mathbb{R}^{n_u} \to \mathbb{R}^{n_G}$. The continuous-time ATPF process at the steady state is described by $0 = f(x,u,\theta)$ where $f(x,u) = (f_{\mathrm{d}}(x,u), f_{\mathrm{a}}(x,u))$ denotes the DAE system. The measurable outputs $y \in \mathbb{R}^{n_y}$ are described by $y = g(x,u;\theta)$. We distinguish constraints on u only, given by $G(u)$, as we can guarantee to satisfy these constraints, whereas constraints $H_i(u,y)$ express desirables and might not always be satisfied due to the unknown plant dynamics. Possible constraints are upper and lower bounds on the gas volume flow rate and the conductivity. The cost function $H_0(u,y)$ describes the control goals, i.e. high phospholipase concentration and activity in the top phase. The steady state values $(u^{\mathrm{ss}}, x^{\mathrm{ss}}, \hat{y}^{\mathrm{ss}})$ provided by the above optimization problem are tracked by an underlying NMPC controller. The dynamics and the output function are parameterized by disturbance parameters θ which allow for online model adaptation. One possible parametrization that is typically used within the MA literature [31, 32, 35] are simple additive corrections on the output equation:

$$g(x,u,\theta) = \tilde{g}(x,u) + a_k + A_k(u - u_k)$$

with zero-order correction a_k and first-order correction A_k summarized by $\theta_k = (A_k, a_k)$. Various alternative formulations of the augmented model are possible and are to be investigated in this project. An important property that has to be satisfied by the augmented model is *model adequacy* [31, 36], which ensures that the augmented model is able to capture the plant dynamics at the steady state up to first-order. To achieve plant optimality, experimental gradient estimation is required which often poses a major challenge when applying MA methods in practice. Directional modifier adaptation (D-MA) techniques thus estimate only a few directional derivatives to reduce the number of experimental evaluations of the true system dynamics. How to choose these *privileged* directions, in which the gradient is estimated, is subject to ongoing research [35, 37–39]. An additional challenge poses the question of how to estimate sensitivities from noisy data [35].
For the ATPF process, only a few state variables can be measured. Therefore, state and parameter estimation needs to be performed. We plan to use Moving Horizon Estimation (MHE), an optimization-based nonlinear method, which takes into account a trajectory of the most recent

measurements $y(t_{n-N}), \ldots, y(t_N)$ to formulate the estimation problem:

$$\begin{aligned} \min_{x(\cdot),\theta} \quad & \pi_{n-N}\left(x(t_{n-N});\theta\right) + \sum_{n-N}^{n} l_k\left(g(x(t_k),u(t_k);\theta) - y(t_k)\right) \\ \text{s.t.} \quad & \dot{x}(t) = f(u_k, x(t);\theta),\, t \in [t_k, t_{k+1}), k = n-N, \ldots, N-1, \end{aligned} \tag{4}$$

with loss $l_k : \mathbb{R}^{n_y} \to \mathbb{R}$, arrival cost $\pi_{n-N} : \mathbb{R}^{n_x} \to \mathbb{R}$, and horizon N. The advantage of the MHE approach is the extremely flexible formulation in terms of an optimization problem: Different noise distributions, as well as varying accuracies can be accounted for by the loss function. For instance, a Gaussian noise assumptions corresponds to a least-squares cost function l_k. In addition, the nonlinearity of the model including disturbance parameters that enter the model nonlinearly are taken into account.

For the steady-state problem (3), a large amount of uncertainty will lie in the estimated parameter θ. In some directions, the sensitivity of the optimization problem with respect to θ may be significant. If the resulting steady state is only optimized with respect to a point estimate $\hat{\theta}$, slight deviations from this value might lead to large deviations in the objective or constraint values. This creates the need to consider the uncertainty explicitly in the optimization. There are two major approaches to incorporate uncertainty: robust optimization [40] and stochastic programming [41]. In robust optimization, one considers a set $\Theta \subset \mathbb{R}^{n_\theta}$ of parameters such that $\theta \in \Theta$ holds with near-certain probability. Such a set may be readily obtained from the MHE problem (4), as for every parameter estimate $\hat{\theta}$ it also supplies an estimated covariance. From these, one could construct Θ as a confidence region around $\hat{\theta}$. In the following we focus on the relationship between input and output at the steady state. Note that, under standard regularity assumptions, the steady state condition and the output model in (3) implicitly define the output as a function of the input, $y = \tilde{F}(u;\theta)$. Using this to eliminate x and y, we obtain a robust steady state from the robustified problem

$$\min_{u} \max_{\theta\in\Theta} H_0(u, \tilde{F}(u;\theta)) \quad \text{s.t.} \quad \begin{cases} \max\limits_{\theta\in\Theta} H_i(u, \tilde{F}(u;\theta)) \leq 0, & i = 1, \ldots, n_H, \\ G(u) \leq 0, \end{cases} \tag{5}$$

which optimizes the worst case objective and ensures constraint satisfaction for all $\theta \in \Theta$. In a stochastic framework, the uncertainties would be modelled by a probability distribution, and the considered problem would be to minimize, e.g., the expected value of the cost functions subject to the constraints holding with a certain probability. Due to its bi-level structure ("minimax"), problem (5) is in this general form intractable. However, if the lower level maximization problems are characterized by specific structures, such as convexity, or belong to a special problem class, they can become tractable. Even if (5) can not be formulated as problem of a simpler class, one may use these classes as building blocks for approximations (possibly conservative) to (5) and then solve these approximated problems directly [42] or in an iterative fashion [43].

While modifier adaptation allows the controller to find optimal steady states based on the behavior of the real process, it does not take into account the trajectory towards the steady state. A powerful form of feedback control for this purpose is model predictive control (MPC) [8], or, more specifically, nonlinear MPC (NMPC) in the context of nonlinear systems. MPC uses a system model to predict trajectories of the system. The control inputs are then found by optimizing over future trajectories by solving an optimal control problem (OCP). This allows one to explicitly incorporate constraints on both states and control inputs into the OCP formulation. The OCP is continously solved in real time to react to deviations from the predicted trajectory. However, standard (nominal) MPC has no explicit model of this uncertainty. This is not sufficient to ensure robust constraint satisfaction, as a trajectory at the boundary of the feasible set can be tipped into infeasibility by a small perturbation. There are two main paradigms for uncertainty-aware MPC: stochastic MPC (SMPC) [44] and robust MPC (RMPC) [45, 46]. In both approaches, knowledge of the uncertainty is included in the

system model. The specific challenges here are (a) the propagation of uncertainty sets through time, and (b) the optimization over feedback policies instead of open loop controls, as knowledge of future feedback (or recourse) needs to be incorporated into the problem formulation to avoid the prediction of unreallistically fast growing uncertainty sets. However standard SMPC and RMPC only consider static uncertainty estimates, in the sense that they are not aware that uncertainty may be reduced by learning about the system. This is the topic of the field of dual control [47, 48]: how can the conflicting objectives of exploration to learn about the system dynamics and exploitation of the obtained knowledge be balanced? This can either be implicit, by approximately encoding the impact of uncertainty and the possibility to learn in the optimization problem formulation, or explicit, by incentivising exploration through additional heuristic terms in the objective function or by small random perturbations of the problem. The privileged directions for gradient estimation in the MA approach can be seen as a form of explicit dual control.

Preliminary Studies. The Systems and Control Laboratory at the Department of Microsystems has many years of experience in the formulation and solution of estimation and control problems for nonlinear dynamic processes based on numerical optimization. This includes the development of highly efficient numerical solvers tailored to optimal control problems, which are implemented in widely used open-source software such as the embedded nonlinear optimal control solver `acados` [6], its predecessor `ACADO` [49], the interior point method `HPIPM` [50], the linear algebra library `BLASFEO` [51], as well as the nonlinear optimization and algorithmic differentiation tool `CasADi` [52]. The algorithms are developed in close feedback with practitioners, with applications ranging from the control of electric motors [53, 54] with sampling times down to the three digit microsecond range, over autonomous car racing [55] to large scale chemical processes [56] and building temperature control [57]. Previous work has focused not only on algorithms for general nonlinear robust optimization [43, 58], but also on the optimal control of uncertain systems [9, 59–61], including efficient software implementations of tailored algorithms [62, 63]. More recently, this also involves the stochastic NMPC of photovoltaic battery systems [64] and most importantly, a currently active DFG project on numerical methods for ellipsoid based and tree sparse robust NMPC formulations (project number 424107692). In ellipsoid based approaches, the uncertainty is described by ellipsoidal sets (tubes) around the nominal trajectory, whereas in scenario-tree formulations [63] one spans a tree of the possible scenarios, based on the assumption of discrete uncertainty sets. First results of the project include a zero-order algorithm for robust NMPC with ellipsoidal tubes [65], and one for optimally choosing linear feedback laws to avoid the the conservativeness of open loop robust optimal control [66]. Regarding compensation of model-plant mismatch and online model adaptation, previous work has focused on optimization-based iterative learning control [7, 10, 67] and online parameter estimation [68, 69]. In particular, it could be shown that for highly constrained systems, plant optimality can be achieved using only zero-order corrections [7].

Research Gap. Up to date there is no industrial application of an ATPF process recorded. To enable the industrial use of a continuous ATPF for the purification of biotechnological products, a controllable ATPF process must be developed that can be operated efficiently by an optimal control strategy. Online measuring capabilities and a structurally adequate model are prerequisites for the development of an adaptive controller that adjusts for model-plant mismatch online and can react to changing operating conditions. This allows for the development of a tailored control algorithm that on the one hand incorporates first principle knowledge of the process, but on the other hand is able to leverage sparse data. More explicitly, the controller should control the separation process with regard to maximum product quality and explicitly account for high measurement inaccuracies, fluctuating input concentrations and model uncertainties. Therefore implementing online monitoring for the continuous ATPF apparatus is essential since it allows a close characterization of the process behavior and thereby the validation of the new model as well as feedback control. The advantages of the developed methodology are its modularity and flexibility, such that it can be easily adjusted for similar processes.

1.2 Project-related publications

1.2.1 Articles published by outlets with scientific quality assurance, book publications, and works accepted for publication but not yet published

[1] L. Jakob, J. Singer, and H. Nirschl. “Importance of gas input in aqueous two-phase flotation (ATPF)”. In: *Chemical Engineering Science* 233 (2021), 116391.

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2 Objectives and work program

2.1 Anticipated total duration of the project

6 years, 3 years in the first period.

2.2 Objectives

The aim of this research project is to establish an *autonomously controlled continuous ATPF process*. To this end, online measuring procedures for the relevant input and output variables are required. Coupled with suitable modeling of the mass transfer within the ATPF apparatus, an NMPC strategy is developed which is able to adapt the model online via zero and first-order model corrections. Model uncertainties, as well as stochastic process and measurement disturbances, are explicitly accounted for within the control and estimation problem formulation. This should result in an innovative separation process that is able to react to fluctuating input variables and to control it with respect to the target variable. Due to the abstraction level of the control problem formulations, the adaptive optimal control concept can be easily adapted to other biotechnological products and biosuspensions.

Establishing a Measurable and Controllable Continuous ATPF Process. For monitoring the continuous ATPF process, online measurements are needed. Implementing flow cells for UV/Vis measurements shall be implemented for continuously recording enzyme concentration and activity. For this, developing an continuous colorimetric enzyme assay is essential. Furthermore, detecting the mixing of the phases by conductivity sensors shall be established. A process control system is to be set up to record the online measurement data, feed it to the control system and pass on setpoint changes to the individual control units.

Modelling of the Continuous ATPF Process. A sufficiently accurate but yet fast and straightforward process model describing the continuous ATPF process in terms of a DAE is developed for the first time. The focus lies on a trade-off between the necessary level of detail in describing the micro-processes and model complexity. The main goal is to develop a validated numerical model

that describes the ATPF process, is sufficiently adaptable to minimize model-plant mismatch under realistic conditions, and handles the relevant control parameters as inputs.

Adaptive Optimal Control Strategy for Continuous ATPF. The aim of this project is a closed-loop control system running an optimization-based controller that uses a model of the ATPF process, which is updated based on measured data. Both for the control part and the estimation part, efficient numerical methods are used that exploit the particular structure of the particular optimization problems at hand. The available measurements are used efficiently for updating model disturbance parameters and additional experimental evaluations of the system dynamics are kept at a minimum. In addition, important aims of the project will be to (a) identify the most important sources of uncertainty and incorporate them into the model, (b) quantify their impact and relevance online via uncertainty estimation, (c) robustify the controller against their impact on both levels, and (d) enabling dual control by explictly encoding the explore-exploit tradeoff in the controller.

2.3 Work program including proposed research methods

Research group 1 (KIT, Karlsruhe) is responsible for the experimental studies (WP 1). Research group 2 (IMTEK, Freiburg) develops the control strategy (WP 3). Both groups jointly develop the process model (WP 2) and work hand in hand while testing the control strategy (WP 1.3). The work program is described below and a time table is given in Table 1.

WP 1: Establishing a Measurable and Controllable Continuous ATPF Process

WP 1.1: Implementing Online Monitoring. For monitoring the ATPF, UV/Vis flow cells are to be installed at the inlets and outlets of the apparatus. By continuously recording the absorption spectra, both the enzyme concentrations and the quantity of impurities can be recorded. To measure the product quality, i.e. the phospholipase activity in the concentrated top phase online, an assay unit must be installed. For this purpose, a second flow cell must also be designed that allows the continuous addition of the reaction solution required for the colorimetric assay. In addition to substrate and cofactors, this solution contains a suitable indicator (e.g. phenol red). The flow rates must be adjusted so that the cleavage of the lecithin and the associated acidification change the color spectrum of the indicator. The decrease in absorbance can be detected by an integrated UV/Vis measurement and thus the phospholipase activity can be quantified. This online reaction chamber should be designed to ensure the shortest possible delay between leaving the ATPF apparatus and detection of phospholipase activity. By 3D printing this reaction chamber, prototypes can be made quickly and easily. In order to be able to detect the phase swirling, the conductivity in the bottom phase should be measured. For this purpose, it is conceivable to integrate a conductivity sensor at one or more points in the ATPF apparatus.

WP 1.2: Characterizing Dynamic Process Behavior. In order to simulate the dynamic process behavior of continuous enzyme fermentations, experiments with different feed compositions will be performed starting from a model suspension (containing phospholipase and optionally relevant impurities). The change of the phospholipase and impurities concentrations in the feed will be simulated both abruptly and in ramps. Firstly, the process response (phospholipase concentration and activity at the outlet) will be recorded for constant control parameters (pump rates and gas flow rates). Likewise, the influence and limits of the control variables are to be determined. For this purpose, the control parameters are varied at constant feed composition and the separation result as well as the phase swirling are analyzed.

WP 1.3: Testing of the Control Strategy. In this work package, the intelligent controller is to be integrated into the continuous ATPF. For this purpose, the connectivity between the online measurement data, the control system and the control units (pumps and mass flow controller) must be established. This is to be done via a suitable process control system (e.g. LabView). Subsequently, the intelligent controller is to be tested by varying the feed composition in a targeted manner, similar to WP 1.2. Here, the two research groups will work closely together to optimally

link the process with the control strategy. After any necessary adjustments and fine-tuning of the controller, the continuous ATPF process is to be confronted with a separation task that is as real as possible. For this purpose, experiments with unknown feed variations are to be carried out.

WP 2: Modelling of the Continuous ATPF Process

WP 2.1: Development of a DAE model. In this work package, the DAE describing the continuous ATPF process as elucidated in Chapter 1.1 is developed. Firstly, all equations describing relevant process variables, as e.g. the enzyme concentration $c_{\mathrm{enz}}(i, j, t)$ are set up. This includes defining suitable expressions for the transport effects visualized in Fig. 5, incorporating literature and preliminary work on ATPE and ATPF [1, 2]. Subsequently, suitable spatial discretization schemes are elaborated, the most straightforward one being a compartment setup as indicated in Fig. 5. At this stage it is also investigated if the problem may possess an analytical solution for the steady state, which may be useful to validate the DAE in the first place. The main challenge in describing the real system lies in the kinetic rates, such as diffusion coefficients or adsorption rates of enzyme onto the bubbles, which may be derived from the literature, but are associated with great uncertainties. In this case, it seems expedient to adjust said kinetic rates to experimental data via, e.g., model identification or Machine Learning algorithms [70]. Either way, the resulting process model requires validation: A thorough comparison to the experimental data from WP 1.2 is elementary to assess the model accuracy achieved. In particular, these experiments might indicate that further effects, like turbulence, back-flow of bubbles from the liquid-liquid interface or the coalescence of bubbles, have to be implemented.

WP 2.2: Disturbance Modelling and Dynamic Disturbance Estimation. We plan to develop and validate different disturbance models for the ATPF process satisfying model adequacy. Furthermore, we aim at analyzing more general necessary and sufficient criteria for model adequacy, as well as for the weaker property of zero-order adaptability. Additional evaluation criterions for the disturbance model might be computational aspects, e.g. cost for evaluating the model and its sensitivities, as well as simplicity, i.e. we would like to estimate as little additional disturbance parameters as possible. Based on the augmented DAE model, an MHE problem is formulated. As part of this work package, a loss function for the MHE problem is designed which takes into account the very different accuracies and error distributions of the available measurements. For instance, high accuracy conductivity measurements are available, whereas measurements of the phospholipase activity are rather noisy and might be corrupted by outliers. At each timestep, the MHE problem provides both state and disturbance parameter estimates $\hat{x}$ and $\hat{\theta}$, together with a matrix covariance estimate, which are used within the control problem formulation, see WP 3.1 and 3.2.

WP 3: Adaptive Optimal Control Strategy for Continuous ATPF Process

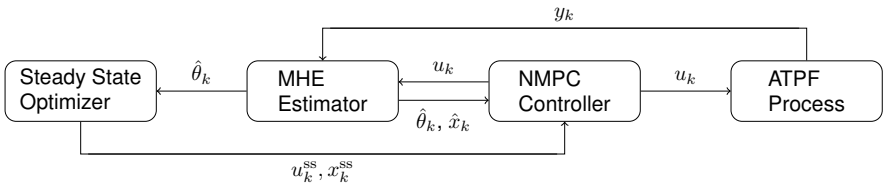

Figure 6: Closed-loop Control System.

WP 3.1: Adaptive Steady-State Optimization and Tracking NMPC. In this work package, we implement the closed-loop control system, which includes the MHE estimator, the steady state control problem and tracking NMPC controller, as well as the plant. A schematic overview is pictured in Fig. 6. The steady state problem and the NMPC controller are based on the augmented DAE model developed in WP 2.1 and 2.2 and use the most up-to-date disturbance parameter es-

timates. Significant delays within the measurement procedure are to be expected and need to be accounted for. The particular problem structure arising from the DAE model is to be exploited and a tailored numerical treatment of the constraints $G(u)$ and $H_i(u, y)$ will be examined.
Implementation, validation and tuning of the closed-loop control system are the main focus of this work package. Evaluation criteria include the computation times for the individual components, the speed of convergence of the estimated disturbance parameters, as well as control performance.

WP 3.2: Uncertainty-Aware Control. The OCP formulations in both layers should incorporate uncertainty aspects of the model dynamics which poses an additional challenge as numerical solution of the resulting problem formulations should be feasible in real-time. The goal of this work package is therefore two-fold, with similar challenges on both levels of the controller: (a) formulate uncertainty-aware optimization problems in the spirit of (5) that are adequate to the relevant uncertainties of the real system. This in close cooperation with WP 1.2 and WP 2.2. (b) find or develop efficient algorithms for this optimization problem. This can involve the identification of specific structures in the optimization problem or computationally tractable approximations.

WP 3.3: Dual Control for Optimal Process Excitation. Combining both the original control objective, as well as the objective of estimating the sensitivities of the true system dynamics leads to a dual control problem. In the presence of active constraints, exact derivative information of the dynamic model is not required in *all* directions. We plan to develop and analyze different strategies for selecting the *privileged directions* [37], in which experimentally obtained derivative information is required. Concepts of dual control can also be effective for the lower level tracking problem. If the NMPC problem is not only robustified against uncertainties, but also includes a simplified model of both future feedback control and the observer, this makes the controller implicitly aware of the explore-exploit tradeoff: trajectories of high objective value will usually be blocked by constraints, such that the controller will push against them. As large uncertainties require a larger backoff to be kept from the constraints, one way to reach better objective values is to reduce the uncertainty, as quantified by the Lagrangian inequality multipliers. The second aim is therefore to find tailored and tractable formulations of the lower level tracking problem, that include models of future feedback and observation, to be able to automatically choose between exploration and exploitation.

Table 1: Gantt chart of the individual work packages.

Work Packages ■ KIT-Karlsruhe ■ IMTEK-Freiburg		**Year 1**				**Year 2**				**Year 3**			
WP 1	**Establishing a Measurable and Controllable Continuous ATPF Process**												
WP 1.1	Implementing Online Monitoring	■	■	■									
WP 1.2	Characterizing Dynamic Process Behavior			■	■	■	■	■					
WP 1.3	Testing of the Control Strategy							◢	◢	◢	◢	◢	◢
WP 2	**Modelling of the Continuous ATPF Process**												
WP 2.1	Development of a DAE model	◢	◢	◢	◢	◢	◢						
WP 2.2	Disturbance Modelling					◢	◢	◢	◢				
WP 3	**Adaptive Optimal Control Strategy for Continuous ATPF Process**												
WP 3.1	Adaptive Steady-State Optimization	■	■	■	■								
WP 3.2	Uncertainty-Aware Control					■	■	■	■				
WP 3.3	Dual Control for Optimal Process Excitation									■	■	■	■

3 Bibliography concerning the state of the art, the research objectives, and the work program

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4 Relevance of sex, gender and/or diversity

Not required

5 Supplementary information on the research context

5.1 Ethical and/or legal aspects of the project

5.1.1 General ethical aspects

Not required

5.1.2 Descriptions of proposed investigations involving experiments on humans or human materials

The project involves no experiments with humans or human material.

5.1.3 Descriptions of proposed investigations involving experiments on animals

The project involves no experiments with animals, or that involve genetic resources.

5.1.4 Descriptions of projects involving genetic resources (or associated traditional knowledge) from a foreign country

The project involves neither genetic resources, nor associated traditional knowledge.

5.1.5 Descriptions of investigations involving dual use research of concern, foreign trade regulations

Not required

5.2 Data handling

The achieved data within the research project are permanently and centrally backed up, which excludes data loss. The research data is available to the participants of the SPP for further research. Data processing is handled according to the recommendation of the National Research Data Infrastructure NFDI4Ing.

5.3 Other information

— N/A —

6 People/collaborations/funding

6.1 Employment status information

The applicants of this project are:

Name	Position	Affiliation
Prof. Dr.-Ing. Hermann Nirschl	Professor (W3), permanent employment	Karlsruhe Institute of Technology
Prof. Dr. Moritz Diehl	Professor (W3), permanent employment	University of Freiburg

6.2 First-time proposal data

— N/A —

6.3 Composition of the project group

Nirschl group:

Name	Employment status	Type of funding
Prof. Dr.-Ing. Hermann Nirschl	Head of the group Process Machines at the KIT	permanent
Dr.-Ing Marco Gleiss	Senior scientist in the group Process Machines at the KIT	permanent
(M. Sc.) N.N.	PhD student	non-permanent

Diehl group:

Name	Employment status	Type of funding
Prof. Dr. rer. nat. Moritz Diehl	Head of the Systems Control and Optimization Laboratory, Albert-Ludwigs-Universität Freiburg	permanent
Dr. Gianluca Frison	Postdoc (20%) at the Systems Control and Optimization Laboratory	non-permanent
(M. Sc.) N.N.	PhD student	non-permanent

6.4 Researchers in Germany with whom you have agreed to cooperate on this project

We will collaborate on this project together with the following researchers from Germany:

Name	Affiliation	Research area
Prof. Dr.-Ing. Thomas Becker	TUM	Brewing and Beverage Technology/ Process Engineering in Biotechnology
Prof. Dr. et Ing. habil. Kerstin Eckert	TU Dresden	Fluid Dynamics/ Transport Processes at Interfaces
Prof. Dr.-Ing. Sergio Lucia	TU Dortmund	Uncertainty-aware Model Predictive Control
Prof. Dr. rer. nat. habil. Sebastian Sager	Univ. Magdeburg	Application Driven methods for Nonlinear Optimization, Mixed Integer Optimal Control
Dr.-Ing. Karin Schwarzenberger	HZDR/ TU Dresden	Interfacial Phenomena/Transport Processes at Interfaces

With the research **group of Prof. Becker** a collaboration on the general interaction of enzymes and gas-water interfaces is intended.
With the research **group of Prof. Eckert** a collaboration addressing the measurement of gas dispersion parameters (bubble size distributions, bubble-surface-area flux) is planned.
With the research **group of Prof. Lucia** an ongoing collaboration on robust model predictive control is established within the control theoretic DFG project number 424107692, and we agreed to collaborate within SPP 2364 on adaptive robust model predictive control formulations.
With the research **group of Prof. Sager** a collaboration is agreed focusing on the development of numerically beneficial differential equation model formulations for particulate processes and on open-source algorithms for the solution of the resulting nonlinear optimal control problems.
With the research **group of Dr. Schwarzenberger** a collaboration is agreed focusing on the adsorption of the enzymes on the bubble surface as well as on the phase stability.

6.5 Researchers abroad with whom you have agreed to cooperate on this project

We will collaborate on this project together with the following researchers from abroad:

Name	Affiliation	Research area
Prof. Dr. Boris Houska	ShanghaiTech University	Robust Optimal Control
Prof. Dr. Gabriele Pannochia	University of Pisa	Offset-free Control, Modifier Adaptation Methods for MPC
Prof. Dr. James B. Rawlings	University of California	Model Predictive Control, Stability

6.6 Researchers with whom you have collaborated scientifically within the past three years

Nirschl group: Prof. Dr. Karl Mandel (Erlangen), Prof. Dr. Gerhard Sextl (Würzburg), Prof. Dr. Bernd Friedrich (Aachen), Prof. Dr. Arno Kwade (Braunschweig), Prof. Dr. Carsten Schilde (Braunschweig), Prof. Dr. Norbert Kockmann (Dortmund), Prof. Dr. Urs Peuker (Freiberg), Prof. Dr. Carsten Schilde (Braunschweig), Prof. Dr. Georg Garnweitner (Braunschweig), Prof. Dr. Alexjandro Franco (Université Picardie), Prof. Dr. Arnulf Latz (Ulm), Prof. Dr. Wolfgang Bessler (Offenburg), Prof. Dr. Robert Kee (Colorado School of Mines)
Diehl group: Dr. Mohammad Abdollahpouri (Gothenburg, Sweden), Prof. Dr. Dirk Abel (Aachen), Dr. Thiva Albin (Zürich, Switzerland), Dr. Sebastian Albrecht (München), Prof. Dr. Angelika Altmann-Dieses (Karlsruhe), Prof. Dr. Jakob Andert (Aachen), Dr. Kai Arras (Stuttgart), Dr. Jonas Asprion (Sulgen, Switzerland), Prof. Dr. Alberto Bemporad (Lucca, Italy), Dr. Daniele Bernardini (Lucca, Italy), Prof. Dr. Joschka Bödecker (Freiburg), Dr. Bernardo Brogliato (Grenoble, France), Prof. Dr. Heinrich Bülthoff (Tübingen), Prof. Dr. Wolfram Burgard (Freiburg), Prof. Dr. Ayman El Badawi (Cairo, Egypt), Prof. Dr. Lorenzo Fagiano (Milano, Italy), Prof. Dr. Rolf Findeisen (Magdeburg), Dr. Stefan Gering (Renningen), Dr. Tobias Geyer (Baden-Dättwil, Switzerland), Prof. Dr. Sebastien Gros (Trondheim, Norway), Prof. Dr. Christoph Hackl (München), Dr. Reza Hashemi (Ludwigshafen), Prof. Dr. Boris Houska (Shanghai, China), Prof. Dr. Frank Hutter (Freiburg), Prof. Dr. Tor Arne Johansen (Trondheim, Norway), Prof. Dr. Colin Jones (Lausanne, Switzerland), Prof. Dr. Tina Kasper (Duisburg/Essen), Prof. Dr. Katharina Kohse-Höinghaus (Bielefeld), Prof. Dr. Sergio Lucia (Dortmund), Dr. Kasper Masschaele (Antwerp, Belgium), Prof. Dr. Martin Mönnigmann (Bochum), Prof. Dr. Ion Necoara (Bucharest, Romania), Prof. Dr. Christopher Onder (Aachen), Prof. Dr. Giuseppe Oriolo (Rome, Italy), Dr. Luigi Palmieri (Stuttgart), Prof. Dr. Panos Patrinos (Leuven, Belgium), Prof. Dr. Stefan Pischinger (Aachen), Prof. Dr. Heinz Pitsch (Aachen), Dr. Rien Quirynen (Boston, USA), Prof. Dr. Johannes Reuter (Konstanz), Prof. Dr. Boris Rohal-Ilkiv (Bratislava, Slovakia), Prof. Dr. Sebastian Sager (Magdeburg), Prof. Dr. Gonzalo Sanchez-Arriaga (Madrid, Spain), Prof. Dr. Riccardo Scattolini (Milano, Italy), Dr. Axel Schild (Gifhorn), Prof. Dr. Bernhard Schölkopf (Tübingen), Prof. Dr. Jan Swevers (Leuven, Belgium), Dr. Son Tong (Leuven, Belgium), Prof. Dr. Quoc Tran-Dinh (Chapel Hill, USA), Dr. Ulrich Vollmer (Reutlingen), Prof. Dr. Christof Wittwer (Freiburg), Prof. Dr. Mario Zanon (Lucca, Italy), Prof. Dr. Melanie Zeilinger (Zürich, Switzerland), Dr. Jia-Jie Zhu (Berlin).

6.7 Project-relevant cooperation with commercial enterprises

— N/A —

6.8 Project-relevant participation in commercial enterprises

— N/A —

6.9 Scientific equipment

At the Institute of Mechanical Process Engineering and Mechanics (KIT), the following equipment can be used to carry out this project: Optical analytical centrifuge (LUMiSizer®), LUMiReader, Batch and continuous ATPF apparatus, Lab pressurized filter cell (BHS Sonthofen), Compressiblity-Permeability cell (C-P cell), Lab-decanter centrifuge (Lemitec GmbH), Vacuum belt filter (BHS Sonthofen), Bucket centrifuge with integrated camera, Sorvall RC2-B ultracentrifuge, 3D laser scanning microscope, Keyence AGILISTA 3200W 3D printer, fluorescence microscope Keyence BZ-8000 with digital image evaluation and scanning electron microscope (SEM) with connection for digital image processing, SAXS camera (small-angle X-ray scattering, Xenocs Xeuss 2.0), Zeiss XRadia Versa 520 (µCT), NMR tomograph (Bruker Avance 200, Bruker Avance 400 WB), high-speed camera Photron Fastcam-X-1024, high-resolution cameras AVT Stingray F-033B and AVT Pike F-100B, particle measurement laboratory (Helos, Accusizer, PCS, BET surface determination, density determination, AcoustoSizer (Colloidal Dynamics), etc.), On-line laser diffraction

(Sympatec GmbH), On-line UV-Vis spectroscopy (Ocean Insight), weighing room with analytical scales, mechanics and electronics workshop.

6.10 Other submissions

An application for funding for this project has not been submitted to any other authority. If I submit such an application, I will inform the German Research Foundation immediately. The liaison lecturer of the Karlsruhe Institute of Technology (KIT) will be informed of the application. I undertake to comply with the rules of good scientific practice. I herewith declare, that the requested funds will not be used for any large-scale research assignments (PoF) at KIT.

7 Requested modules/funds

7.1 Basic Module

7.1.1 Funding for staff

Nirschl group:

Number	Description	Duration	Funding
1	Research Assistant, Dipl.-Ing./ M.Sc. (University) TV-L E13	36 months	215.100 €
1	Technician TV-L E9	6 months	26.400 €
1	Student Assistant (B. Sc.) 40 h/month	20 months	12.768 €
		Total	**254.268 €**

Research Assistant (36 month TV-L E13) – The research assistant with a major in process engineering/chemical engineering will work on the project over a total period of 3 years. Her/his tasks include the experimental studies addressing the continuous ATPF. Including the implementation of online measurement techniques, characterizing the process behavior, modelling the separation process and finally testing the control strategy.
Technician (7 month TV-L E9) – The technician supports the research assistant in experimental investigations. This includes the planning (CAD constructions) and installation of apparatus, especially measurement equipment. He helps to design the flow chambers for UV/Vis measurements and enzyme assay. He will be also involved in any possible changes of the ATPF tank as well as in implementing the process control system. Besides he will be in charge of the availability and maintenance of the experimental equipment.
Student assistant (20 month, 40 h/month) – The student assistant with bachelor degree supports the research assistant in simple but time-consuming tasks, such as calibrating pumps, offline analysis of the product (phospholipase) containing top phase after the ATPF-experiment. In addition, the student assistant's field of activity includes the creation of evaluation routines to evaluate the influence of material properties and process parameters to characterize the process behavior.

Diehl group:

Number	Description	Duration	Funding
1	Research Assistant, Dipl.-Ing./ M.Sc. (University) TV-L E13	36 months	215.100 €
1	Student Assistant 40 h/month	20 months	13.680 €
		Total	**228.780 €**

Research Assistant (36 month TV-L E13) – For the development of the desired numerical methods and software one person with background in mathematics or control engineering and solid programming skills is required. The research assistant will work on the project over a total period of 3 years.

Student assistant (20 month, 40 h/month) – The student assistant supports the research assistant in implementing the methods, testing and benchmarking the algorithms.

7.1.2 Direct project costs

7.1.2.1 Equipment up to € 10,000, software and consumables

Nirschl group:

Equipment	Description	Costs
Chemicals	Two-phase system (polymers and salts) and enzymes (phospholipases)	5.000 €
Consumer materials	Cuvettes, pipettes, hoses, etc.	2.000 €
Equipment for online analytic	Two UV/Vis Spectrophotometers,	2x 6.000 €
	Conductivity sensors	1.000 €
	Total	**20.000 €**

Cemicals are needed for the experimental studies, to prepare a model suspension. Large amounts of polymers (e.g. polyethylene glycols) and salts (e.g. citrate) are needed to build the two-phase system. The biosuspension is reconstituted by dissolving spray-dried enzyme powder. This is mainly phospholipase, but also other enzymes such as amylase to generate multicomponent systems.
Consumables are required for offline measurements and experiment preparation.
Analytical instruments are necessary to set up online monitoring. An UV/Vis spectrophotometer is needed to measure the enzyme and impurity concentration. Another is needed to detect enzyme activity during the online assay. Conductivity sensors are needed to detect phase turbulence.

7.1.2.2 Travel expenses

Nirschl group:

Description	Number	Costs for each
Scientific conferences, conference participation (domestic)	3	800 €
Scientific conferences, conference participation (abroad)	2	2.000 €
Participation in SPP project meetings	3	300 €
	Total	**7.300 €**

Diehl group:

Description	Number	Costs for each
Scientific conferences, conference participation (domestic)	3	800 €
Scientific conferences, conference participation (abroad)	2	2.000 €
Participation in SPP project meetings	3	300 €
	Total	**7.300 €**

7.1.2.3 Visiting researchers (excluding Mercator Fellows)

— N/A —

7.1.2.4 Expenses for laboratory animals

— N/A —

7.1.2.5 Other costs

— N/A —

7.1.2.6 Project-related publication expenses

Nirschl group: Two open access publications (2.000 € each publication) **4.000 €**
Diehl group: Two open access publications (2.000 € each publication) **4.000 €**

7.1.3 Instrumentation

7.1.3.1 Equipment exceeding € 10,000

— N/A —

7.1.3.2 Major instrumentation exceeding € 50,000

— N/A —

7.2 Module Temporary Position for Principal Investigator

— N/A —

7.3 Module Replacements

— N/A —

7.4 Module Temporary Substitute for Clinicians

— N/A —

7.5 Module Mercator Fellow

— N/A —

7.6 Module Project-Specific Workshops

— N/A —

7.7 Module Public Relations

— N/A —