High-throughput screening of optimal process conditions using model predictive control

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Abstract

Modern biotechnological laboratories are equipped with advanced parallel mini-bioreactor facilities that can perform sophisticated cultivation strategies (e.g. fed-batch or continuous) and generate significant amounts of measurement data. These systems require not only optimal experimental designs that find the best conditions in very large design spaces, but also algorithms that manage to operate a large number of different cultivations in parallel within a well-defined and tightly constrained operating regime. Existing advanced process control algorithms have to be tailored to tackle the specific issues of such facilities such as: a very complex biological system, constant changes in the metabolic activity and phenotypes, shifts of pH and/or temperature, and metabolic switches, e.g. by product induction, to name a few. In this work we implement a model-predictive control (MPC) approach based framework to demonstrate: 1) the challenges in terms of mathematical model structure, state and parameter estimation, and optimization under highly nonlinear and stiff constraints in biological systems, 2) the adaptations required to enable its application in High Throughput Bioprocess Development (HTBD), and 3) the added value of MPC implementations when operating parallel mini-bioreactors aiming to maximize the biomass concentration while coping with hard constrains on the Dissolved Oxygen Tension profile.Modern biotechnological laboratories are equipped with advanced parallel mini-bioreactor facilities that can perform sophisticated cultivation strategies (e.g. fed-batch or continuous) and generate significant amounts of measurement data. These systems require not only optimal experimental designs that find the best conditions in very large design spaces, but also algorithms that manage to operate a large number of different cultivations in parallel within a well-defined and tightly constrained operating regime. Existing advanced process control algorithms have to be tailored to tackle the specific issues of such facilities such as: a very complex biological system, constant changes in the metabolic activity and phenotypes, shifts of pH and/or temperature, and metabolic switches, e.g., by induction of product formation, to name a few.

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Modern biotechnological laboratories are equipped with advanced parallel mini-bioreactor facilities that can perform sophisticated cultivation strategies (e.g. fed-batch or continuous) and generate significant amounts of measurement data. These systems require not only optimal experimental designs that find the best conditions in very large design spaces, but also algorithms that manage to operate a large number of different cultivations in parallel within a well-defined and tightly constrained operating regime. Existing advanced process control algorithms have to be tailored to tackle the specific issues of such facilities such as: a very complex biological system, constant changes in the metabolic activity and phenotypes, shifts of pH and/or temperature, and metabolic switches, e.g., by induction of product formation, to name a few.

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1 Introduction

Production of recombinant proteins using microbial cell factories has seen a dramatic increase over the last decades (Huang et al., 2012). However, finding optimal process conditions for the production of a new protein still remains a challenge, since the number of strains and possible operating conditions to be tested can be very large (Neubauer et al., 2013). The introduction of Mini-Bioreactors (MBR), and in particular their combination with liquid handling stations (LHS), have partially alleviated these problems by enabling high-throughput experiments. Especially when combined with modeling and simulation tools, such platforms were successfully applied for model based experimental re-design (Cruz Bournazou et al., 2017),

strain and process characterization (Anane, García, et al., 2019; Anane, Sawatzki, et al., 2019; Sawatzki et al., 2018) or conditional screening of mutants (Hans et al., 2020; Hemmerich et al., 2019).

However, these systems still have problems when it comes to scale-up, because of the inhomogeneous cultivation conditions in large-scale bioreactors, which are not as pronounced in such small scale systems (working volume < 20 mL) (Nadal-Rey et al., 2021; Neubauer & Junne, 2016). Hence, proper experiments must be designed so that findings are also applicable in a larger scale. The control of the substrate feeding e.g., offers a simple way to mirror certain heterogeneous process conditions. In this regard, bolus feeding with pulses has proven to be a simple but powerful approach to model the effect of inhomogeneous mixing in large-scale bioreactors (Anane, Sawatzki, et al., 2019). Organisms with a high substrate affinity, as e.g. *Escherichia Coli* (*E. coli*), exhibit high oxygen consumption rates. In systems with a small oxygen transfer coefficient (k_La) such as MBRs, this leads to rapid dynamics of DOT changes, causing large obstacles, that are difficult to overcome.

Hence, operating such MBR systems using LHS with limited online and at-line measurements available is still considered a major challenge (Morschett et al., 2021). In this respect, the current contribution builds on our previous work, where we successfully implemented a framework for high-throughput cultivation with conditional screening capabilities (Hans et al., 2020). Avoidance of DOT limitation is a crucial part in optimal operation of such devices, since pulse-based feeding typically leads to drastic stress responses, fast changes in DOT due to fast substrate uptake and elevated levels of corresponding genes (Schweder et al., 1999) as well as elevated secretion of several unwanted byproducts like acetate and reduced biomass yield (Bylund et al., 1998). As already mentioned, with the pulse-based feeding approach used in this study, violation of this constraint might easily happen. After applying a pulse, the DOT drops sharply, as the cells start to consume glucose at a high rate. This may even lead to oxygen limitation and to the induction of anaerobic responses (Schweder et al., 1999). Later, after depletion of the glucose, the DOT rises again to the pre-pulse value. In this bolus-feed based setting, conventional (PID) controllers would fail because they can only react after a

glucose pulse has been added and thus a constraint could have been violated shortly afterwards. This is especially true for strongly nonlinear systems like the one presented in this study. Hence, predictive control algorithms like Model Predictive Controllers (MPC) are required to avoid such conditions. MPC is an advanced control approach based on a dynamic model of the system which computes the control inputs aiming to minimize a given cost function and satisfy predefined constraints (Rawlings et al., 2017). While widely applied in chemical engineering, MPC has only found relatively few applications in bioprocess engineering (see e.g. the comprehensive review by Mears et al., 2017). One of the first (linear) MPC applications was presented by Kovárova-Kovar et al. to maximize product formation (Kovárová-Kovar et al., 2000). Further examples exist for different cases as e.g. slow growing mammalian cells (Ashoori et al., 2009), yeast (Chang et al., 2016) and bacterial cultivations (Del Rio-Chanona et al., 2016; Ulonska et al., 2018). Another approach is to perform set point tracking to follow a predefined trajectory (Craven et al., 2014; Zhang & Lennox, 2004).

The main challenges for the application of linear MPC result from the high nonlinearities and dynamics of biological systems (Shin et al., 2019). Therefore, in recent years the application of nonlinear MPC (NMPC) has become more and more prominent (Schwenzer et al., 2021). MPC is a powerful approach but is limited by the accuracy of the model and by the data provided to make optimal decisions. In our specific case, i.e. at the early stage of cultivation, the MPC framework should be able to find an optimal feeding trajectory in real-time time despite optimal model parameters are not known beforehand and the scarce data on the strains under investigation. Hence, it is of great importance to have robust adaptive methods that can perform well under these difficult conditions. The counterpart of MPC, Moving Horizon Estimation (MHE) is a powerful tool to estimate states and parameters of the model and is an excellent complement to MPC (Hille et al., 2020). Using MHE for state and parameter re-estimation has been proposed for process engineering for some time and various examples can be found in the literature (Hedengren & Eaton, 2017; Jabarivelisdeh et al., 2020; Zavala et al., 2008). The reader is further referred to Elsheikh et al., 2021 for a comprehensive review.

We will discuss in this contribution how we tackled several issues which are commonly faced in these constrained and highly perturbed fed-batch cultivations in MBRs as (i) the discontinuity of the feeding regime, i.e., the bolus type addition of glucose to the reactors; (ii) system delay to the input, which make predictive control essential to avoid constraint violation; (iii) the differences in the dynamics of the timescales of the system of differential equations, particularly regarding growth of biomass and the DOT, where the time dynamics differ by orders of magnitudes and thus lead to a very stiff system; (iv) the different measurement frequencies (high for DOT and low for biomass, glucose, and acetate); and (v) the uncertainty in the parameter values of the model, which are unknown prior to the cultivation and might be only based on rough knowledge about the strains. Thus, in a limited amount of time, the MHE needs to solve the highly nonlinear and non-convex parameter estimation problem with sufficient accuracy for the MPC to compute inputs that guide the real process to the expected results.

To demonstrate the advantages and challenges of our approach, the production of Elastin Like Proteins (ELPs) in *E. coli* was chosen as an interesting case-study. ELPs are derived from natural tropoelastin and are promising examples of biocompatible, self-assembling and flexible high-performance materials with a great potential for various applications (Huber et al., 2015; Huber et al., 2022; MacEwan & Chilkoti, 2014). The properties of the protein depend on the sequence composition, i.e. the amino acids in the repetitive pentapeptide sequence, as well as the length of the protein (Huber et al., 2014; Schreiber et al., 2019). In order to develop specific characteristics, large clone libraries with different strains are created, for which optimal process conditions for production are yet to be identified. Due to the diverse use of individual amino acids at the fourth position of the repeating sequence and a limited set of core amino acids used (especially proline and valine) the optimization of ELP production depends on multiple parameters such as feed strategies and oxygen supply. Therefore, this case-study is highly interesting to test the MHE/MPC framework to find an optimal feeding trajectory without prior knowledge of the strains.

2 Materials and Methods

2.1 High throughput bioprocess development facility

All experiments were conducted on our high-throughput bioprocess development platform. The platform comprises two liquid handling stations (Freedom Evo 200, Tecan, Switzerland; Microlab Star, Hamilton, Switzerland), a mini bioreactor system (48 BioReactor, 2mag AG, Munich, Germany) and a Synergy MX microwell plate reader (BioTek Instruments GmbH, Bad Friedrichshall, Germany). The MBRs have a working volume of 8-12 mL and are equipped with fluorometric sensor spots (PreSens Precision Sensing GmbH, Regensburg, Germany) to measure DOT and pH. The LHS performs feeding by adding defined volumes of concentrated glucose solution to the reactors (bolus feeding) in a predefined timeframe. Sampling is automatically performed in regular intervals and the optical density at 600 nm (OD_{600}), fluorescence (as measure for the product concentration) as well as concentrations of glucose and acetate are automatically analyzed at-line on our high-throughput bioprocess development platform. The reader is referred to Haby et al., 2019 for a detailed description of the facility, the sampling and feeding procedure.

2.2 Strain and cultivation conditions

All experiments were carried out with *E. coli* BL21(DE3), carrying the plasmid pET28-NMBL-eGFP-TEVrec-$(V_2Y)_{15}$-His, expressing a recombinant fusion protein of ELP and eGFP, under the isopropyl-β-D-thiogalactopyranosid (IPTG) inducible *lac*UV5-promoter. Detailed information about the plasmid can be found in Huber (Huber et al., 2014) and Schreiber (Schreiber et al., 2019). The linkage of the actual target protein, ELP to an eGFP allows a simple non-invasive measurement of the protein concentration. The amount of product, i.e. ELP is calculated based on a conversion factor from the fluorescence measurements which was determined in previous studies. All chemicals were purchased from either Roth, VWR or Merck if not stated otherwise. For the first preculture, 10 mL LB medium, containing 16 g L^{-1} tryptone, 10 g L^{-1} yeast extract and 5 g L^{-1} NaCl, were directly inoculated with 100 µL cryostock and cultured in a 125 mL Ultra Yield flask (Thomson Instrument Company, USA) sealed with

an AirOtop enhanced flask seal (Thomson Instrument Company, USA) for 5 h at 37°C and 200 rpm in an orbital shaker (Adolf Kühner AG, Birsfelden, Switzerland). The second pre-culture was performed with 25 mL EnPresso B (Enpresso GmbH, Berlin, Germany) medium with 9 U L^{-1} Reagent A. The composition of the EnPresso B is the same as the main medium used, besides the glucose polymer. This system allows for constant glucose release from the polymer in a fed-batch like manner in a 250 mL Ultra Yield flask, and thus prevents overfeeding even in the preculture. After 12 h, while in exponential growth phase, appropriate volumes of the pre-culture were used to inoculate the MBRs to an OD_{600} of 0.25. The minimal medium in the actual bioreactors consisted as derived from Glazyrina et al., 2010 of mineral salt medium, containing (per L): 2 g Na_2SO_4, 2.468 g $(NH_4)_2SO_4$, 0.5 g NH_4Cl, 14.6 g K_2HPO_4, 3.6 g NaH_2PO_4 × 2 H_2O, 1 g $(NH_4)_2$-H-citrate and 1 mL antifoam (Antifoam 204, Sigma). Before inoculation, the medium was supplemented with 2 mL L^{-1} trace elements solution, 2 mL L^{-1} MgSO4 solution (1.0 M) and kanamycin to a final concentration of 50 mg L^{-1}. The trace element solution comprised (per L): 0.5 g $CaCl_2$ × 2 H_2O, 0.18 g $ZnSO_4$ × 7 H_2O, 0.1 g $MnSO_4$ × H_2O, 20.1 g Na-EDTA, 16.7 g $FeCl_3$ × 6 H_2O, 0.16 g $CuSO_4$ × 5 H_2O, 0.18 g $CoCl_2$ × 6 H_2O, 0.132 g Na_2SeO_3 × 5 H_2O, 0.12 g Na_2MoO_4 × 2 H_2O, 0.725 g $Ni(NO_3)_2$ × 6 H_2O. In all bioreactor cultivations, the initial glucose concentration for the batch phase was 3 g L^{-1}. At the end of the batch phase, indicated by a sharp rise of DOT, the MHE/MPC controller was started to fit the model to recent available data and start calculating an optimal feeding regime. Feeding was performed by adding glucose pulses (solution with a concentration of 200 g L^{-1} glucose) every 10 min by the LHS. This type of feeding exposes the cells to a high glucose concentration for a short time, which is characterized by a steep drop in DOT. After the cells have consumed the glucose, the DOT rises again, resulting in the characteristic oscillating DOT profile. These oscillations come from the fact that DOT drops steeply after the addition of a feeding pulse, as soon as the cells begin to take up glucose. After all glucose is depleted in the pulse period, the DOT rises back to its pre-pulse value. Immediately after the pulse is added, the DOT drops so sharply, that a violation of the constrain of having at least 30 % DOT in the reactors can quickly occur. The pulse feed trajectory for the cultivations which were not controlled by MPC was

calculated according to (1) and then integrated over each pulse duration (10 min) to find the volume to be added within a single pulse.

$$F(t) = \frac{\left(\frac{\mu_{set}}{Y_{X/S}} + q_m\right) * X * V}{S_i} * exp(\mu_{set} * t) \quad (1)$$

Here F [L h^{-1}] describes the feed rate over the time t [h], μ_{set} [h^{-1}] the specific growth rate, $Y_{X/S}$ [g g^{-1}] the yield coefficient of glucose per biomass, q_m [g g^{-1} h^{-1}] the specific glucose consumption for maintenance (0.02 g g^{-1} h^{-1} were used in this study), S_i [g L^{-1}] the glucose concentration in the feed and X [g L^{-1}] as well as V [L] respectively the biomass concentration and volume at the end of the batch phase. All liquid additions as well as the sampling volumes are stored in the database, so that the current volume and corresponding dilution effects can be always calculated accurately.

2.3 Sampling / Analytics

Throughout the cultivation, DOT and pH were measured online, using the photometric sensors at the bottom of the MBRs. Due to the position of the sensors and because the sensors were calibrated under process conditions, gas bubbles in this process do not represent a disturbance of the sensors. For the other state variables, samples were taken every 20 min from one of the replicate columns and directly inactivated with dried 2 M NaOH in 96 well plates and stored at 4°C until further analysis. After collection of 3 columns of samples, the plate was automatically transferred to the Hamilton robot for at-line analysis of OD_{600}, fluorescence, glucose, and acetate concentration. The reader is referred to Haby et al., 2019 for a detailed description of the analysis process.

2.4 Principles of the MHE/MPC framework

The objective of this study is to find optimal process conditions for strains with little a prior knowledge of their growth behavior. Therefore, a model-based framework was created consisting of an MHE and an MPC part: a moving horizon estimator to estimate the parameters and initial states of the model based on recent measurements; and a model predictive control

part to calculate an optimal feeding profile for each condition. Since the strain under investigation was cultivated under 6 different conditions in three replicates, a total of $N_r = 18$ mini bioreactors were used. Each of the bioreactors $r \in R = \{1, \dots, N_r\}$ can be described by the nonlinear dynamics:

$$\dot{x}_r(t) = f(x_r(t), u_r(t), \theta_r)$$
$$x_r(t_0) = x_{0,r} \tag{2}$$

The dynamic states are denoted by the vector of ODEs $\dot{x}_r$ and include biomass, the substrate glucose, DOT, product (measured via fluorescence), bioreactor volume as well as acetate. The control inputs for each mini bioreactor are $u_r \in R^{N_u}$, while $\theta_r \in R^{N_\theta}$ denotes the unknown parameter vector of the reactors and cultivation conditions and $x_{0,r}$ are the initial conditions for each reactor. The inputs are applied as time-discrete bolus-type pulses. This leads to a highly discontinuous operation with jumps in the volume and concentrations of the other state variables. Thus, after each pulse, the concentrations are recalculated based on the previous concentrations and the pulse volume. The time-series evolution of the denoted states can be described by a system of ordinary differential equations (ODE). The ODE system exhibits dynamics in very different timescales, especially regarding biomass growth and DOT, leading to a very stiff system. Since the dynamics of DOT are usually very fast compared to the other dynamics, they can be expressed in a reduced form as an algebraic equation and thereby reduce the stiffness of the system significantly building a differential-algebraic system of equations (DAE) (Duan et al., 2020). Since the actual DOT (DOT_a) can be only measured with a first order delay, the measured DOT (DOT_m) is also considered as a state variable, taking the response time of the sensor into account. The model has 6 differential states, 1 control input and 18 parameters in total. A complete overview about the equations of the macro kinetic growth model and the meaning of the respective parameters can be found in Kim et al., 2022.

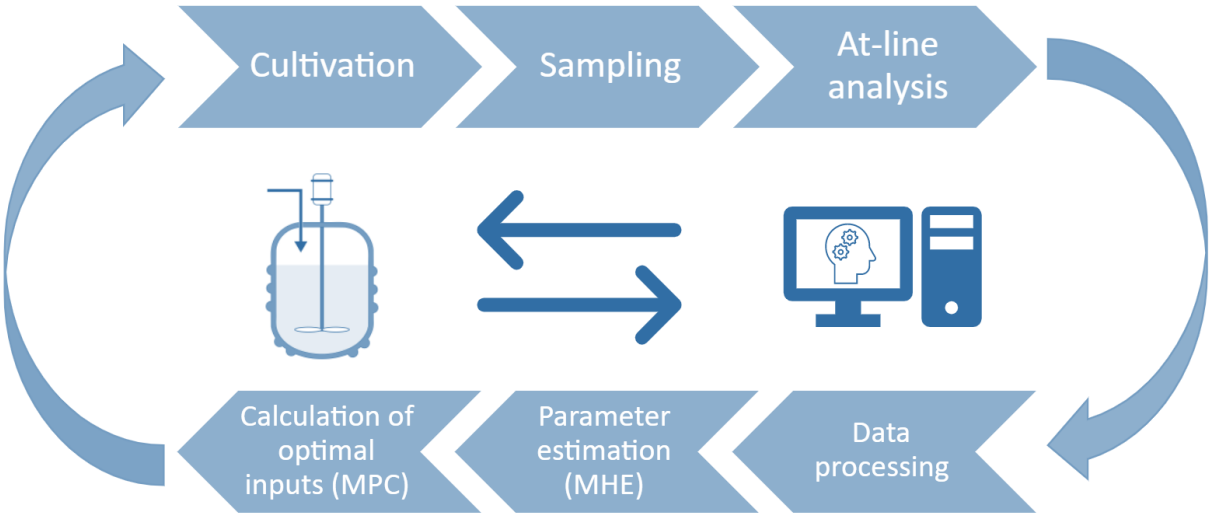


Figure 1: Flowchart of the MHE/MPC framework. During the cultivation, samplings are taken in regular intervals, processed for at-line analysis and used for subsequent parameter estimation and MPC calculations.

An overview about the workflow is depicted in Figure 1. Following this procedure, the parameter set is continuously updated and used for MPC calculations. Considering the N_{MHE} last measurements, the optimization problem for obtaining a new set of parameters and initial states of the new horizon window can be written as:

$$\min_{\theta, x_{0,r}} \frac{1}{2}\left\|x_{0,r} - x_{0,r,old}\right\|^2_{W_x} + \frac{1}{2}\|\theta - \theta_{old}\|^2_{W_P} + \sum_{k=0}^{N_{MHE}} \frac{1}{2}\|h(x_r(t), u_r(t), \theta) - y_{meas}(t)\|^2_{W_y} \tag{3}$$

s.t.

$$\dot{x}_r(t) = f(x_r(t), u_r(t), \theta) \tag{4}$$

$$\theta_{min} \leq \theta \leq \theta_{max}$$

The objective function is composed of the following parts: The estimate for the states at the initial point of the window $x_{0,r}$ and the prior estimate for that state $x_{0,r,old}$ as well as the difference between the current parameter vector θ and the previous parameter estimate vector θ_{old}. The final optimal parameter set is denoted as $\hat{\theta}$. The last term is the summed difference between the predicted outputs $h(\cdot)$ as function of the states $x_r(t)$, the inputs $u_r(t)$ and parameters θ and the available measurements $y_{meas}(t)$. $\|x\|^2_{W_i} = x^T W_i x$ denotes the squared norm, weighted by the matrix W_i. The subscript r indicates the respective set of MBR

replicates. θ_{min} and θ_{max} refer to the lower and upper boundaries of the parameter vector. The penalty on the parameter changes in the objective function ($\theta - \theta_{old}$) assures that, in each iteration, the parameters are not adapted too much considering their previous values.

The MPC calculates optimal inputs to maximize biomass at the end of the feeding phase, considering that the DOT should not drop below a predefined threshold of 30 %. A detailed description of the MPC and its mathematical formulation can be found in Kim et al., 2022. The general problem can be written as follows:

$$\min_{u_r} - W_M X_r(t + N_{MPC}\Delta t) - W_L \sum_{k=0}^{N_{MPC}-1} X_r(t + k\Delta t) \tag{5}$$

s.t.

$$\begin{gathered} \dot{x}_r(t) = f\left(x_r(t), u_r(t), \hat{\theta}\right) \\ x_r(t_0) = \hat{x}_{0,r} \\ DOT_r(t) \geq 30\ \%, \qquad u_r(t) \geq 3\ \mu\text{L} \end{gathered} \tag{6}$$

The optimization problem is composed of two parts: the terminal cost term (also called Mayer term) $W_M X_r(t + N_{MPC}\Delta t)$ and the stage-cost term (also called Lagrangian term) $W_L \sum_{k=0}^{N_{MPC}-1} X_r(t + k\Delta t)$. W_M and W_L denote the weighting matrices for the respective terms. $\hat{x}_{0,r}$ refers to the last point of the previous MHE timeframe, which is in turn the first element of the new MPC frame. X_r is the biomass, which should be maximized in the control horizon N_{MPC} and Δt is the timeframe between two pulses. The system is subject to the constraints of keeping the DOT above 30 % and to pipette at least 3 µL in every pulse. In every cycle, the MHE fits the model to the recent measured values by updating the parameter values and predicting new values for the initial state of the MPC. With the updated parameters, the MPC is started and calculates new inputs until the end of the feeding phase and beginning of induction. By using an efficient nonlinear program solver (IPOPT) and parallelization, the total calculation time for MPC for 24 bioreactors does not exceed the 10 minutes control interval. A schematic overview about the workflow is depicted in Figure 2.

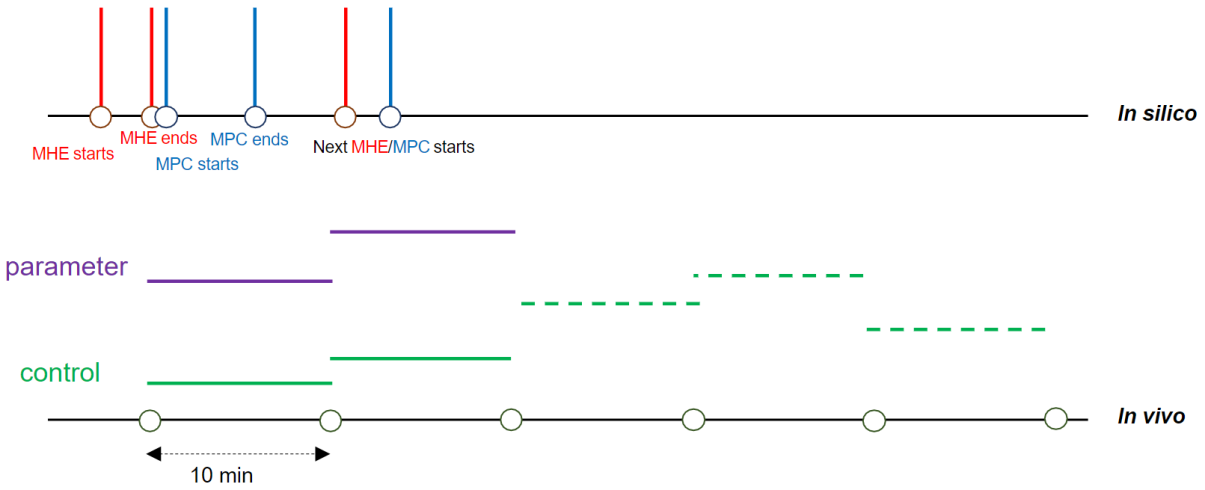


Figure 2: Overview about the MPC workflow. Glucose pulses (the inputs) are given every 10 min as indicated by the circles. The current control inputs for each interval are represented by the green solid lines. Every 10 min, the MHE updates the model parameter (purple lines) by fitting the model to the most recent data. The updated model is used for the MPC to calculate new feeding inputs until induction. The updated inputs are represented by the dashed green lines.

The MHE/MPC framework implemented using an adapted version of do-mpc (Lucia et al., 2017) was compared with a conventional screening approach, which tested the boundaries of the design space to identify optimal cultivation conditions as shown in Table 1 (A-D). The growth rates and respective induction strengths chosen for the conventional approach are based on initial screening experiments and indicate that a possible optimum is in this range (data not shown).

Table 1: Overview about the experimental layout. Depicted are the 6 experimental layouts, stating if MPC was applied (+) or not (-) and in case the DOT constraint, the growth rate, and the induction strengths. The first 4 designs comprise the boundaries of the design space and are based on early screening results, while the latter 2 were controlled by MPC.

Exp. setting	MPC (DOT constraint)	μ_{set} [h^{-1}]	IPTG [mM]
A	-	0.15	0.05
B	-	0.30	0.05
C	-	0.15	2.00
D	-	0.30	2.00
E	+ (30 %)	Controlled by MPC	0.05
F	+ (30 %)	Controlled by MPC	2.00

3 Results

3.1 Identifying optimal process conditions and avoiding adverse DOT limitations

Finding optimal cultivation conditions is a significant task during the development of a new biotechnological process. Many biotechnological processes depend on aerobic conditions, since oxygen limitation would lead to a substantial change of the internal metabolism and lead to a considerable stress response of the cells (Schweder et al., 1999). To reduce the number of necessary experiments until optimal process conditions are found, our previously available HT cultivation system has been extended by an innovative MPC approach. The MPC framework tries to find an optimal feeding rate according to the last generated data, but it has to take into account that the DOT does not drop below 30 % and the system cannot pipette less than 3 µL. Considering these constraints, an optimal feeding profile was found, which maximizes the biomass at the end. Figure 3 shows such an optimal trajectory at one iteration, where the color-coded constraints were considered.

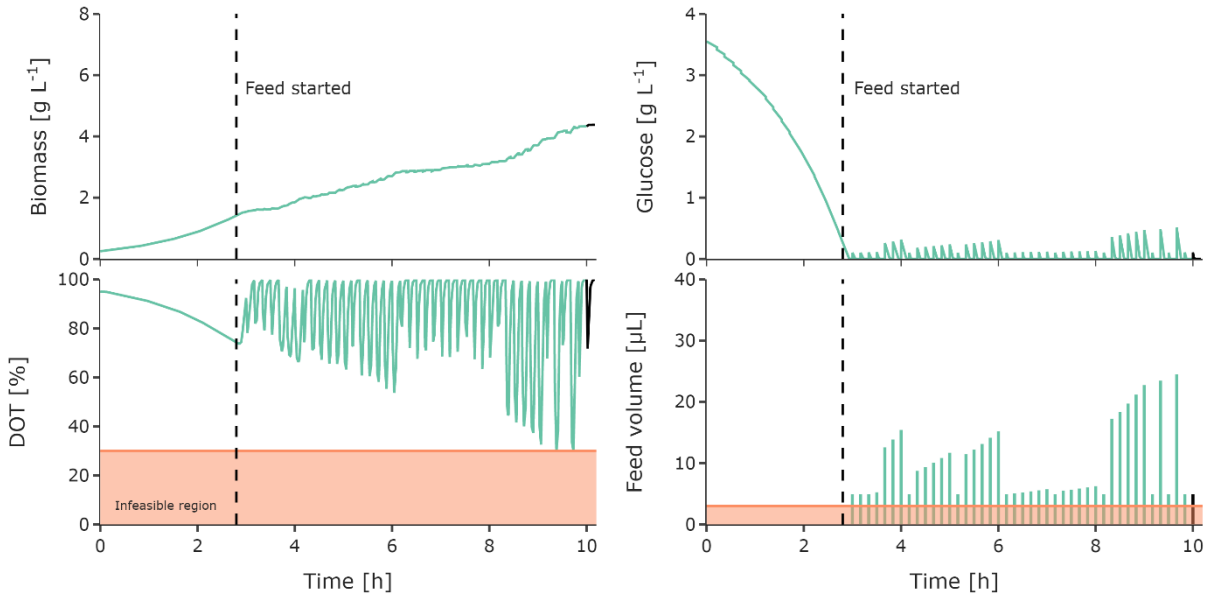


Figure 3: Optimal trajectory avoiding infeasible regions. Shown is a possible trajectory calculated by the MPC framework to obtain high biomass with a pulsed based feeding. Indicated are the infeasible regions (colored areas) as are low levels of oxygen (< 30 %) or low pipetting volumes (< 3 µL). The new suggested input from the MPC is indicated in black.

The MPC framework optimized the feeding trajectory to maximize biomass at the end of the feeding rate while complying with constraints, using the parameters obtained from fitting the model to the data which are measured. Accurate estimates for the parameters of the underlying

dynamical model are essential to ensure truly optimal inputs for the real process. The MHE updated the parameter values every 10 min via fitting the model to the most recent 4 h of the process. Figure 4 shows a parameter estimation which was performed after the experiment to show the capabilities of the model to describe the data and find good parameter values. This emphasizes that the model and framework used are capable of estimating good parameter values that can be used in the MPC framework to calculate optimal feeding. While biomass is slightly overestimated by the model during the fed-batch phase, there is good agreement for substrate and the measured DOT signal in the batch phase, even though the fitting accuracy deteriorated during the induction phase. Acetic acid is underestimated by the model, especially in the beginning of the feeding phase, but the measured values are still in a low range and the prediction error is small. Underestimating the acetate could lead to wrong predictions of the substrate, since acetate is inhibiting biomass growth.

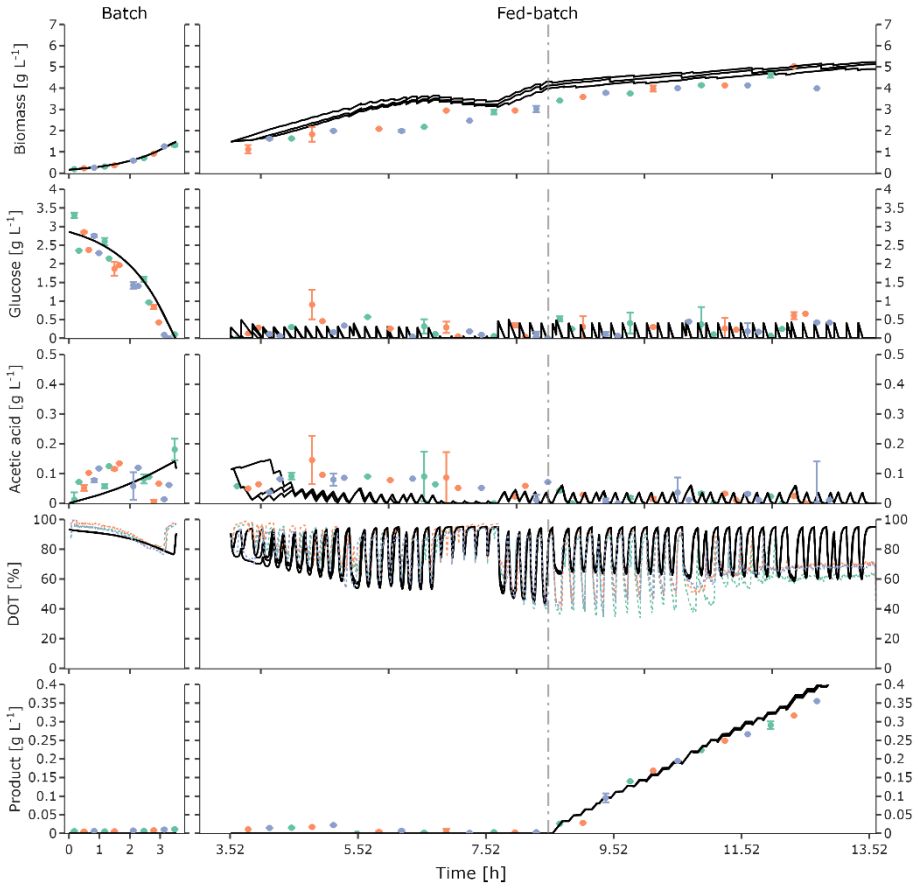


Figure 4: *A posteriori* parameter estimation. Shown is the output of the parameter estimation after the process was performed from 3 replicate reactors (colored dots, each color representing one of the triplicate bioreactors). Note the differences in time scales between batch and fed-batch phases. In this setting, product refers to the ELP-eGFP fusion protein (which was measured via Fluorescence and converted to g L^{-1} with previously calibrated conversion factor). The dash-dotted line at around 8 h indicates the point of induction.

The MPC framework generated a good feeding trajectory to reach a high biomass, while considering that the constraints are not violated. The results of the experiments following the layout of Table 1 are depicted in Figure 5. After a batch phase of around 4 h, typically detected by the sudden increase in the DOT signal, the feed and MPC controller were started. For the conventional approach, 4 experiments (in triplicates) according to the experiments A-D in Table 1 were fed with a predefined feed at a μ_{set} of 0.30 h^{-1} or 0.15 h^{-1}. The other two experiments (also in triplicates) were fed with individual feeds (Figure 6 E and F) which were calculated from the MPC controller and updated every 10 min. The reactors with the higher feed rate reached higher biomass values at the end of the process compared to the reactors with the lower feed rate (Figure 6 A and C) and therefore also higher values for the product concentration as depicted in Figure 5. However, especially after induction, the DOT signal drops below the threshold of 30 % in those reactors and cells entered overflow metabolism, which is also indicated by glucose accumulation and higher levels of acetate. Induction strength has only minor impact on the production. The cultivations with the higher IPTG concentration showed slightly higher product concentration levels normalized to the biomass than the cultivations with lower IPTG. In the reactors, which were controlled by the MPC framework (Figure 6 E and F), the biomass reached comparable levels between the high and the low predefined feeding rate as shown in Figure 6A and B. All reactors which were controlled by the MPC framework satisfied the constraint of having oxygen levels over 30 %. Glucose accumulation was only observed after induction in those reactors with the high induction level and acetate remained almost constant during the course of the cultivation. Product concentration levels were also as high as in the cultivations with the predefined feed. As a result, the biomass obtained was similar to the high μ_{set} but without violating the DOT constrains. This is an increase of approx. 50 % compared to the non-controlled cultivations that stayed within bounds was achieved.

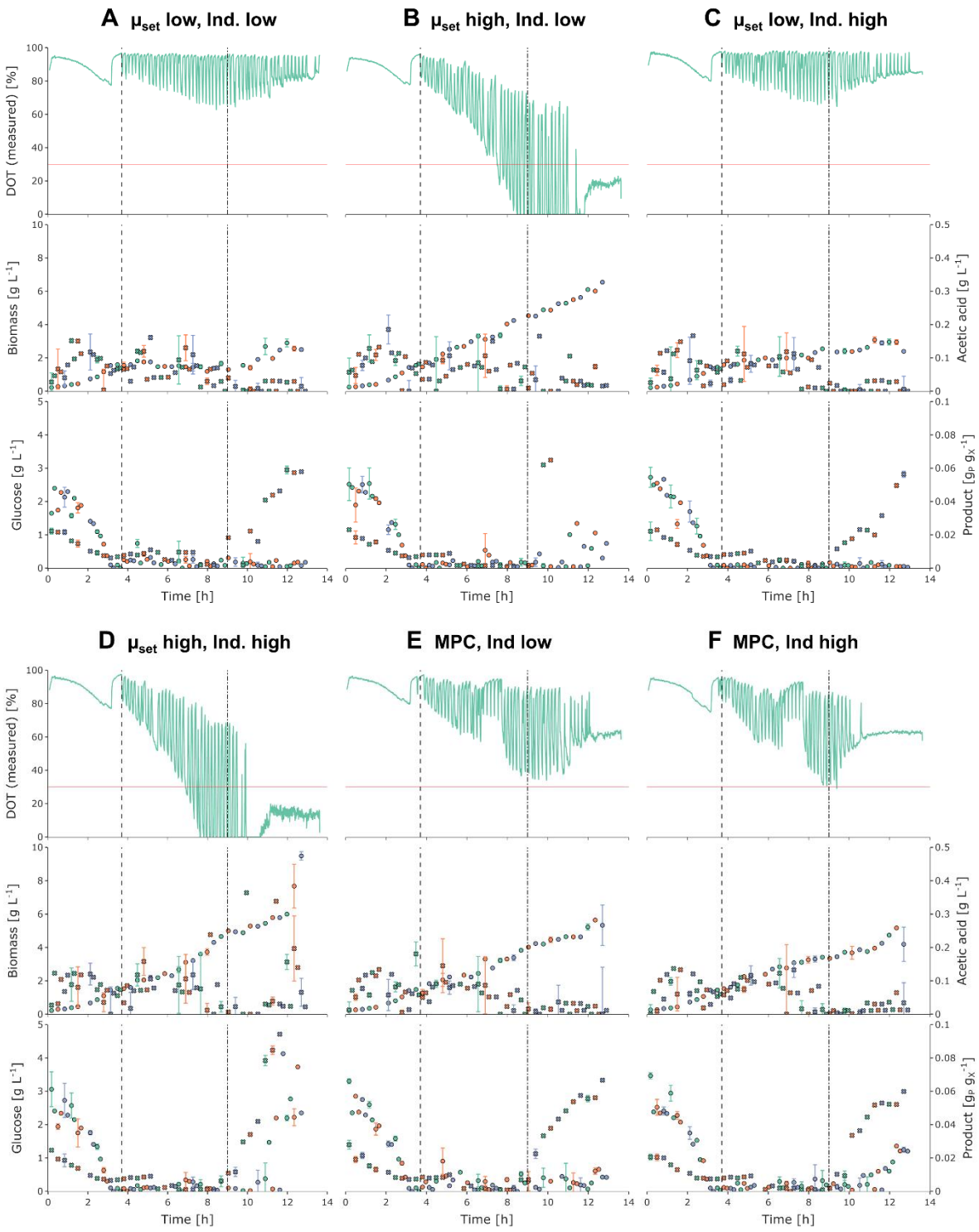
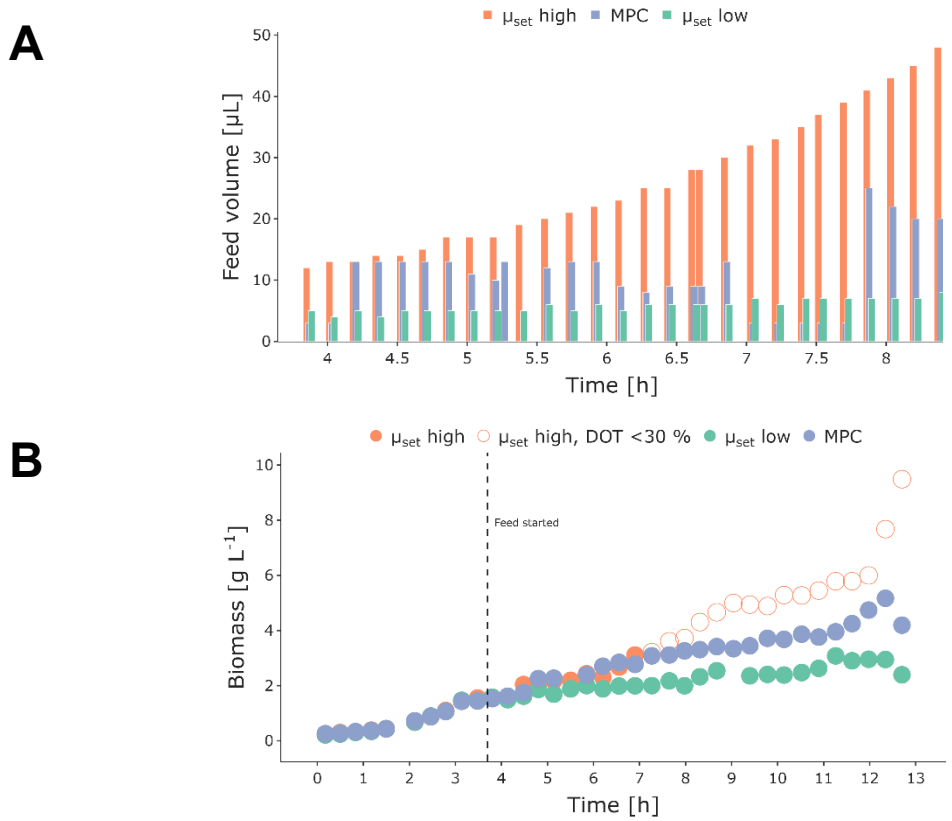


Figure 5 Results from the first cultivation. In the figures A-D are the cultivations depicted with low (0.15 h^{-1}) and high (0.3 h^{-1}) feeding rate as well with low (0.02 mM) and high (2 mM) induction with IPTG. The part figures E and F show the comparison of the processes which are controlled by MPC, again with the low and high induction. Depicted are the measurements for measured DOT, biomass (circles), glucose (circles), acetate (x) and product per biomass (x). The dashed vertical line indicates the start of the feed and the dash-dotted vertical line the start of the induction.



364 **Figure 6: Comparison of the feed profiles and the biomass.** Depicted are the different bolus feed volumes at
365 each feeding time during the exponential feeding phase (A) and the measured cell dry weight for the high and the
366 low predefined growth rate as well as for the cultivation which was controlled by the MPC framework (B). The
367 dashed line indicates the start of the feeding. Open circles indicate when a cultivation has violated the constraint of
368 having at least 30 % DOT.

369

370 # 4 Discussion

371 ## 4.1 Optimal process control with limited a priori knowledge

372 In this study, we have extended our existing automated high-throughput bioprocess
373 development platform with an MPC framework that allows new *E. coli* strains, about which little
374 prior knowledge is available, to be cultured at their maximum growth capacities. By using online
375 and various at-line measurements, it is possible to measure the key state variables at high
376 frequency and generate sufficient data to fit our mechanistic model of the organism to these
377 data. Unlike previous examples of MPC in bioprocesses, the parameter values of the model
378 do not need to be known in advance, but are adaptively fitted to the measured values during
379 the model (Jabarivelisdeh et al., 2020). This made it possible to determine better cultivation
380 conditions in a single run than would be the case with classical feeding profiles. However,
381 further tuning of the framework is still needed to further optimize the optimal feeding trajectory.
382 Furthermore, we show that this MPC based control of the process is necessary to meet the

constraints (DOT > 30 %) even though a bolus-based feeding is used. A classical PID controller, on the other hand, could not respond until a glucose pulse was given, which could lead to a violation of the constraint in this system (Santos et al., 2012). In addition, Kager et al. compared stability and performance of a PID controller with MPC and found that the PID controller often cannot cope with the nonlinear dynamics and is unstable, and MPC furthermore achieves better performance. e.g., higher yield (Kager et al., 2020). In addition, a PID controller cannot handle nonlinear process constraints such as oxygen limitation. These constraints can only be met with the help of model knowledge in the form of mathematical optimization.

4.2 MHE/MPC guides to optimal process conditions

Operating a high-throughput MBR system is a challenging task and violation of several process constraints might easily happen (Hemmerich et al., 2018). This is especially true when screening a new strain for optimal process conditions, where the biological parameters are unknown before the experiment. The MPC controller successfully managed to maintain the process within the predefined bounds. The approach was compared to a classical approach with predefined feeding rates: Two different feed rates were applied to the process which are often applied in bioprocesses of *E. coli*: μ_{set} = 0.15 h^{-1} or μ_{set} = 0.3 h^{-1}, respectively. The low feeding rate did not achieve the high biomass outputs that would be possible with the strain. On the other hand, cultivating the cells with the higher feed-rate led to significant oxygen limitation as can be seen in Figure 5 and Figure 6. An adaptive computation of the optimal profile was necessary to maximize biomass concentration without violating process constraints.

Even though the feeding calculated with the MPC led to significantly better results than with the predefined feed, the optimal feeding profile was not achieved. This is mainly due to plant-model mismatches and inaccuracies of the measurements, which have great influence on the simulation outcome (Nagy & Braatz, 2004). Due to the uncertainties of the parameters which are currently not considered in the nominal MPC, the actual optimal feeding rate could have

been higher. Further tuning of the MPC framework, which would make it more aggressive and penalize constraint violation less, could lead to higher yields.

4.3 Control under uncertainty

In particular, uncertainties inherent in the model as well as uncertainties in the parameters lead to sub-optimal feeding profiles. Especially after induction, the model is less accurate to describe the process. The use of hybrid models could improve model predictions and reduce dependence on individual parameter values (Stosch et al., 2014). However, this requires very large data sets to train such models well. In addition, they are sometimes worse at generalizing for unknown strains. Furthermore, the use of data-driven approaches such as PCA (Thombre et al., 2019) could be supported. In contrast, other approaches in MPC such as multi-stage MPC or stochastic MPC would likely predict more cautious feeding rates so that they do not violate constraints even in the presence of large uncertainties (Lucia et al., 2013).

5 Conclusion and outlook

Finding optimal experimental conditions in early bioprocess development is time consuming and laborious. Even though the combination of liquid handling stations and MBR have decreased the bottleneck in the screening phase, it is still not easy to find optimal process conditions which yield e.g. high biomass or product concentration without violating predefined constraints which might be adverse to the process under investigation. However, cultivating bacterial strains at their maximum capabilities while fulfilling the constraints is essential for a fast and robust bioprocess development framework. We have described how an MPC approach based on a macro-kinetic growth model can be successful to maintain DOT constraints while maximizing biomass production in the exponential growth phase. Hence, within a single parallel run it is possible to identify close to optimal process conditions. Using an adaptive approach like MHE to estimate states and parameters can support the MPC to deliver optimal control inputs. However, the current framework is limited by the uncertainties in the parameters, the model structure, and the time evolution of the system dynamics. Other

implementations are suggested, as e.g. also consider a Kalman Filter, to deal with these uncertainties and plant-model mismatches to ensure a sufficiently accurate parameter estimation and optimal control.

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Conflict of Interest

The authors declare that there is no conflict of interests.

References

Anane, E., García, Á. C., Haby, B., Hans, S., Krausch, N., Krewinkel, M., Hauptmann, P., Neubauer, P., & Cruz Bournazou, M. N. (2019). A model-based framework for parallel scale-down fed-batch cultivations in mini-bioreactors for accelerated phenotyping. *Biotechnology and Bioengineering*, *116*(11), 2906–2918. https://doi.org/10.1002/bit.27116

Anane, E., Sawatzki, A., Neubauer, P., & Cruz Bournazou, M. N. (2019). Modelling concentration gradients in fed-batch cultivations of E. coli - towards the flexible design of scale-down experiments. *Journal of Chemical Technology & Biotechnology*, *94*(2), 516–526. https://doi.org/10.1002/jctb.5798

Ashoori, A., Moshiri, B., Khaki-Sedigh, A., & Bakhtiari, M. R. (2009). Optimal control of a nonlinear fed-batch fermentation process using model predictive approach. *Journal of Process Control*, *19*(7), 1162–1173. https://doi.org/10.1016/j.jprocont.2009.03.006

Bylund, F., Collet, E., Enfors, S.-O., & Larsson, G. (1998). Substrate gradient formation in the large-scale bioreactor lowers cell yield and increases by-product formation. *Bioprocess Engineering*, *18*(3), 171. https://doi.org/10.1007/s004490050427

Chang, L., Liu, X., & Henson, M. A. (2016). Nonlinear model predictive control of fed-batch fermentations using dynamic flux balance models. *Journal of Process Control*, *42*, 137–149. https://doi.org/10.1016/j.jprocont.2016.04.012

Craven, S., Whelan, J., & Glennon, B. (2014). Glucose concentration control of a fed-batch mammalian cell bioprocess using a nonlinear model predictive controller. *Journal of Process Control*, *24*(4), 344–357. https://doi.org/10.1016/j.jprocont.2014.02.007

Cruz Bournazou, M. N., Barz, T., Nickel, D. B., Lopez Cárdenas, D. C., Glauche, F., Knepper, A., & Neubauer, P. (2017). Online optimal experimental re-design in robotic parallel fed-batch cultivation facilities. *Biotechnology and Bioengineering*, *114*(3), 610–619. https://doi.org/10.1002/bit.26192

Del Rio-Chanona, E. A., Zhang, D., & Vassiliadis, V. S. (2016). Model-based real-time optimisation of a fed-batch cyanobacterial hydrogen production process using economic model predictive control strategy. *Chemical Engineering Science*, *142*, 289–298. https://doi.org/10.1016/j.ces.2015.11.043

Duan, Z., Wilms, T., Neubauer, P., Kravaris, C., & Cruz Bournazou, M. N. (2020). Model reduction of aerobic bioprocess models for efficient simulation. *Chemical Engineering Science*, *217*, 115512. https://doi.org/10.1016/j.ces.2020.115512

Elsheikh, M., Hille, R., Tătulea-Codrean, A., & Krämer, S. (2021). A comparative review of multi-rate moving horizon estimation schemes for bioprocess applications. *Computers & Chemical Engineering*, *146*, 107219. https://doi.org/10.1016/j.compchemeng.2020.107219

Glazyrina, J., Materne, E.-M., Dreher, T., Storm, D., Junne, S., Adams, T., Greller, G., & Neubauer, P. (2010). High cell density cultivation and recombinant protein production with Escherichia coli in a rocking-motion-type bioreactor. *Microbial Cell Factories*, *9*, 42. https://doi.org/10.1186/1475-2859-9-42

Haby, B., Hans, S., Anane, E., Sawatzki, A., Krausch, N., Neubauer, P., & Cruz Bournazou, M. N. (2019). Integrated Robotic Mini Bioreactor Platform for Automated, Parallel Microbial Cultivation With Online Data Handling and Process Control. *SLAS Technology*, *24*(6), 569–582. https://doi.org/10.1177/2472630319860775

Hans, S., Haby, B., Krausch, N., Barz, T., Neubauer, P., & Cruz Bournazou, M. N. (2020). Automated Conditional Screening of Multiple Escherichia coli Strains in Parallel Adaptive Fed-Batch Cultivations. *Bioengineering*, *7*(4). https://doi.org/10.3390/bioengineering7040145

Hedengren, J. D., & Eaton, A. N. (2017). Overview of estimation methods for industrial dynamic systems. *Optimization and Engineering*, *18*(1), 155–178. https://doi.org/10.1007/s11081-015-9295-9

Hemmerich, J., Noack, S., Wiechert, W., & Oldiges, M. (2018). Microbioreactor Systems for Accelerated Bioprocess Development. *Biotechnology Journal*, *13*(4), e1700141. https://doi.org/10.1002/biot.201700141

Hemmerich, J., Tenhaef, N., Steffens, C., Kappelmann, J., Weiske, M., Reich, S. J., Wiechert, W., Oldiges, M., & Noack, S. (2019). Less Sacrifice, More Insight: Repeated Low-Volume Sampling of Microbioreactor Cultivations Enables Accelerated Deep Phenotyping of Microbial Strain Libraries. *Biotechnology Journal*, *14*(9), e1800428. https://doi.org/10.1002/biot.201800428

Hille, R., Brandt, H., Colditz, V., Classen, J., Hebing, L., Langer, M., Kreye, S., Neymann, T., Krämer, S., Tränkle, J., Brod, H., & Jockwer, A. (2020). Application of Model-based Online Monitoring and Robust Optimizing Control to Fed-Batch Bioprocesses. *IFAC-PapersOnLine*, *53*(2), 16846–16851. https://doi.org/10.1016/j.ifacol.2020.12.1204

Huang, C.-J., Lin, H., & Yang, X. (2012). Industrial production of recombinant therapeutics in Escherichia coli and its recent advancements. *Journal of Industrial Microbiology & Biotechnology*, *39*(3), 383–399. https://doi.org/10.1007/s10295-011-1082-9

Huber, M. C., Jonas, U., & Schiller, S. M. (2022). An Autonomous Chemically Fueled Artificial Protein Muscle. *Advanced Intelligent Systems*, 2100189. https://doi.org/10.1002/aisy.202100189

Huber, M. C., Schreiber, A., Olshausen, P. von, Varga, B. R., Kretz, O., Joch, B., Barnert, S., Schubert, R., Eimer, S., Kele, P., & Schiller, S. M. (2015). Designer amphiphilic proteins as building blocks for the intracellular formation of organelle-like compartments. *Nature Materials*, *14*(1), 125–132. https://doi.org/10.1038/NMAT4118

Huber, M. C., Schreiber, A., Wild, W., Benz, K., & Schiller, S. M. (2014). Introducing a combinatorial DNA-toolbox platform constituting defined protein-based biohybrid-materials. *Biomaterials*, *35*(31), 8767–8779. https://doi.org/10.1016/j.biomaterials.2014.06.048

Jabarivelisdeh, B., Carius, L., Findeisen, R., & Waldherr, S. (2020). Adaptive predictive control of bioprocesses with constraint-based modeling and estimation. *Computers & Chemical Engineering*, *135*, 106744. https://doi.org/10.1016/j.compchemeng.2020.106744

Kager, J., Tuveri, A., Ulonska, S., Kroll, P., & Herwig, C. (2020). Experimental verification and comparison of model predictive, PID and model inversion control in a Penicillium chrysogenum fed-batch process. *Process Biochemistry*, *90*, 1–11. https://doi.org/10.1016/j.procbio.2019.11.023

Kim, J. W., Krausch, N., Aizpuru, J., Barz, T., Lucia, S., Neubauer, P., & Cruz Bournazou, M. N. (2022). *Model predictive control and moving horizon estimation for adaptive optimal bolus feeding in high-throughput cultivation of E. coli.* https://doi.org/10.48550/arXiv.2203.07211

Kovárová-Kovar, K., Gehlen, S., Kunze, A., Keller, T., Däniken, R. von, Kolb, M., & van Loon, A. P. (2000). Application of model-predictive control based on artificial neural networks to optimize the fed-batch process for riboflavin production. *Journal of Biotechnology*, *79*(1), 39–52. https://doi.org/10.1016/S0168-1656(00)00211-X

Lucia, S., Finkler, T., & Engell, S. (2013). Multi-stage nonlinear model predictive control applied to a semi-batch polymerization reactor under uncertainty. *Journal of Process Control*, *23*(9), 1306–1319. https://doi.org/10.1016/j.jprocont.2013.08.008

Lucia, S., Tătulea-Codrean, A., Schoppmeyer, C., & Engell, S. (2017). Rapid development of modular and sustainable nonlinear model predictive control solutions. *Control Engineering Practice*, *60*, 51–62. https://doi.org/10.1016/j.conengprac.2016.12.009

MacEwan, S. R., & Chilkoti, A. (2014). Applications of elastin-like polypeptides in drug delivery. *Journal of Controlled Release : Official Journal of the Controlled Release Society*, *190*, 314–330. https://doi.org/10.1016/j.jconrel.2014.06.028

Mears, L., Stocks, S. M., Sin, G., & Gernaey, K. V. (2017). A review of control strategies for manipulating the feed rate in fed-batch fermentation processes. *Journal of Biotechnology*, *245*, 34–46. https://doi.org/10.1016/j.jbiotec.2017.01.008

Morschett, H., Tenhaef, N., Hemmerich, J., Herbst, L., Spiertz, M., Dogan, D., Wiechert, W., Noack, S., & Oldiges, M. (2021). Robotic integration enables autonomous operation of laboratory scale stirred tank bioreactors with model-driven process analysis. *Biotechnology and Bioengineering*, *118*(7), 2759–2769. https://doi.org/10.1002/bit.27795

Nadal-Rey, G., McClure, D. D., Kavanagh, J. M., Cornelissen, S., Fletcher, D. F., & Gernaey, K. V. (2021). Understanding gradients in industrial bioreactors. *Biotechnology Advances*, *46*, 107660. https://doi.org/10.1016/j.biotechadv.2020.107660

Nagy, Z. K., & Braatz, R. D. (2004). Open-loop and closed-loop robust optimal control of batch processes using distributional and worst-case analysis. *Journal of Process Control*, *14*(4), 411–422. https://doi.org/10.1016/j.jprocont.2003.07.004

Neubauer, P., Cruz Bournazou, M. N., Glauche, F., Junne, S., Knepper, A., & Raven, M. (2013). Consistent development of bioprocesses from microliter cultures to the industrial scale. *Engineering in Life Sciences*, *13*(3), 224–238. https://doi.org/10.1002/elsc.201200021

Neubauer, P., & Junne, S. (2016). Scale-Up and Scale-Down Methodologies for Bioreactors. In C.-F. Mandenius (Ed.), *Bioreactors* (Vol. 13, pp. 323–354). Wiley-VCH Verlag GmbH & Co. KGaA. https://doi.org/10.1002/9783527683369.ch11

Rawlings, J. B., Mayne, D. Q., & Diehl, M. (2017). *Model predictive control: Theory, computation, and design* (2nd edition). Nob Hill Publishing.

Santos, L. O., Dewasme, L., Coutinho, D., & Wouwer, A. V. (2012). Nonlinear model predictive control of fed-batch cultures of micro-organisms exhibiting overflow metabolism: Assessment and robustness. *Computers & Chemical Engineering*, *39*, 143–151. https://doi.org/10.1016/j.compchemeng.2011.12.010

Sawatzki, A., Hans, S., Narayanan, H., Haby, B., Krausch, N., Sokolov, M., Glauche, F., Riedel, S. L., Neubauer, P., & Cruz Bournazou, M. N. (2018). Accelerated Bioprocess Development of Endopolygalacturonase-Production with Saccharomyces cerevisiae Using Multivariate Prediction in a 48 Mini-Bioreactor Automated Platform. *Bioengineering (Basel, Switzerland)*, *5*(4). https://doi.org/10.3390/bioengineering5040101

Schreiber, A., Stühn, L. G., Huber, M. C., Geissinger, S. E., Rao, A., & Schiller, S. M. (2019). Self-Assembly Toolbox of Tailored Supramolecular Architectures Based on an Amphiphilic Protein Library. *Small*, *15*(30), e1900163. https://doi.org/10.1002/smll.201900163

Schweder, T., Krüger, E., Xu, B., Jürgen, B., Blomsten, G., Enfors, S.-O., & Hecker, M. (1999). Monitoring of genes that respond to process-related stress in large-scale bioprocesses. *Biotechnology and Bioengineering*, *65*(2), 151–159. https://doi.org/10.1002/(SICI)1097-0290(19991020)65:2<151::AID-BIT4>3.0.CO;2-V

Schwenzer, M., Ay, M., Bergs, T., & Abel, D. (2021). Review on model predictive control: an engineering perspective. *The International Journal of Advanced Manufacturing Technology*, *117*(5-6), 1327–1349. https://doi.org/10.1007/s00170-021-07682-3

Shin, S., Venturelli, O. S., & Zavala, V. M. (2019). Scalable nonlinear programming framework for parameter estimation in dynamic biological system models. *PLoS Computational Biology*, *15*(3), e1006828. https://doi.org/10.1371/journal.pcbi.1006828

589 Stosch, M. von, Oliveira, R., Peres, J., & Feyo de Azevedo, S. (2014). Hybrid semi-parametric modeling in process
590 systems engineering: Past, present and future. *Computers & Chemical Engineering*, *60*, 86–101.
591 https://doi.org/10.1016/j.compchemeng.2013.08.008
592 Thombre, M., Krishnamoorthy, D., & Jäschke, J. (2019). Data-driven Online Adaptation of the Scenario-tree in
593 Multistage Model Predictive Control. *IFAC-PapersOnLine*, *52*(1), 461–467.
594 https://doi.org/10.1016/j.ifacol.2019.06.105
595 Ulonska, S., Waldschitz, D., Kager, J., & Herwig, C. (2018). Model predictive control in comparison to elemental
596 balance control in an E. coli fed-batch. *Chemical Engineering Science*, *191*, 459–467.
597 https://doi.org/10.1016/j.ces.2018.06.074
598 Zavala, V. M., Laird, C. D., & Biegler, L. T. (2008). A fast moving horizon estimation algorithm based on nonlinear
599 programming sensitivity. *Journal of Process Control*, *18*(9), 876–884.
600 https://doi.org/10.1016/j.jprocont.2008.06.003
601 Zhang, H., & Lennox, B. (2004). Integrated condition monitoring and control of fed-batch fermentation processes.
602 *Journal of Process Control*, *14*(1), 41–50. https://doi.org/10.1016/S0959-1524(03)00044-1
603