Time Scale Analysis and Optimization of a Continuous Microbial Bioprocess

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Abstract
Continuous bioprocesses are widely used for quantitative strain characterization and industrial fermentations aiming for high space time yields and constant productivity. However, due to the presence of different biochemical reaction dynamics these processes can exhibit slow system behavior under certain conditions. This leads to extended start up times to reach a targeted steady state and impedes to detect and act on process disturbances. We applied a model-based approach to determine the time scale of a continuous Corynebacterium glutamicum bioprocess utilizing a lignocellulosic waste stream in dependence of operational parameters. Time scales were derived by Jacobian linearization of the nonlinear process model and used as an input for multi-objective process optimization. Hereby, operational points with high productivity as well as fast system response upon process disturbances could be obtained. In this way, model-based time scale analysis delivers valuable input for the design of efficient and robust bioprocesses.

Keywords: Bioprocess, dynamic modelling, time scale analysis, multi-objective optimization

1. Introduction
Model-based methods enable a targeted development of bioprocesses. Especially for continuous processes, quantitative understanding is important to select stable operational points. Besides of its relevance in biomanufacturing, continuous cultivation processes are also an important experimental method for the characterization of microbial strains. A three-residence-times rule of thumb is commonly used to reach a hypothetical steady state approximation of 95 % and to derive the corresponding process characteristics. However, this assumption is based on non-reactive tracer experiments and is only proven to be applicable to non-autocatalytic reactions (Heinrichs and Schneider, 1980; Zhang et al., 2007). For different chemical (Zhang et al., 2007) and biochemical systems (Lachmann and Schneider, 1983; Sonnleitner and Hahnemann, 1994) slow system behaviour was reported in the literature, showing a higher number of hydraulic residence times needed to reach a targeted steady state. Methods for time scale analysis based on eigenvalue analysis are well-established in the fields of systems biology (Kremling, 2013) and chemical engineering (Isaac et al., 2013). However, time scale analysis is so far not systematically included in bioprocess design applications. To show the potential and possible effects of time scale analysis within bioprocess development, a continuous C. glutamicum bioprocess utilizing the lignocellulosic waste stream spent sulfite liquor is analyzed in this work. C. glutamicum is a platform organism with high industrial importance and a wide potential product spectrum (Becker et al., 2018). Time scales of
this example process are analyzed in dependence of operational parameters and used as input for process design. In the first part of this work, the applied process model and the methodology for time scale analysis and model-based process design are outlined. Then, the effect of operational parameters on the time scale is shown. The practical relevance of time scale analysis for bioprocess design applications is discussed subsequently, with a focus on system response upon process disturbances.

2. Material and Methods

2.1. Dynamic Process Model

A dynamic process model describing the growth of wild-type *C. glutamicum* ATCC 13032 on spent sulfite liquor was used for simulations. The spent sulfite liquor composition of this study is typical for softwood pulping (Niemelä and Alén, 1999). A general vector form of a dynamic system of mass concentrations $c$, inputs $u$, parameters $\theta$ and disturbances $d$ is shown in Eq. (1).

\[
\dot{c} = f(c, u, d, \theta, t)
\]  

The model describes biomass (X) growth on the two main carbon substrates glucose (S\(_1\)) and mannose (S\(_2\)), as well as conversion of xylose (S\(_3\)) without detectable contribution to biomass formation. The ODEs describing mass concentrations (g L\(^{-1}\)) of biomass $c_X$ and sugar states $c_{S,1}$, $c_{S,2}$, $c_{S,3}$ in a CSTR with constant reactor volume $V_R$ (L) are shown in Eq. (2) and Eq. (3).

\[
\dot{c}_X = \left(\sum_{i=1}^{3} \frac{q_{S,i} Y_{XS,i}}{V_R} - \frac{c_{I,SSL}}{g \cdot L^{-1}}\right) c_X
\]

\[
\dot{c}_{S,i} = \frac{q_{S,in}}{V_R} \left( c_{S,in} - c_{S,i} \right) - q_{S,i} c_X
\]

Sugar uptake $q_{S,i}$ (g S\(_i\) g\(^{-1}\) h\(^{-1}\)) is governed by competitive interaction between different sugars and a non-competitive inhibitory effect (inhibition constant $K_{I,SSL}$ in g L\(^{-1}\)) of spent sulfite liquor concentration $c_{I,SSL}$ (g L\(^{-1}\)), see Eq. (4). Biomass growth and sugar uptake are linked by constant yield coefficients $Y_{XS,i}$ (g X g\(^{-1}\) S\(_i\)). The model parameters were previously determined using experimental data (Sinner et al., 2019). All calculations were performed in MATLAB R2017b (The MathWorks, Inc., United States).

\[
q_{S,i} = \frac{q_{S,max,i} c_{S,i}}{K_{S,i}(1 + \sum_{j=1}^{3} \frac{c_{S,j} + c_{I,SSL}}{K_{I,SSL}})}
\]

2.2. Time Scale Analysis

For time scale analysis, the Jacobian matrix $J$ is used to linearize the nonlinear vector function $f$ around a steady state operating point of interest, see Eq. (5). Hereby, $\Delta$ denotes a deflection of the process variables around the steady state values $\Delta\text{eq}$. The entries $j_{ij}$ of the Jacobian are calculated according to Eq. (6).

\[
\dot{\Delta} = J \Delta
\]

\[
J_{ij} = \frac{\partial f_i}{\partial c_j}\bigg|_{\Delta = \Delta\text{eq}}
\]

By decomposing the Jacobian its eigenvalues $\lambda_\alpha$ are derived. The absolute value of the real part (Re) of the eigenvalue describes the reciprocal time scale $\tau_\alpha$ (h) of a mode, see Eq. (7). The largest time scale $\tau_{max}$ was selected as the dominating time scale among $\tau_\alpha$. 

\[
\frac{1}{\tau_\alpha} = \text{Re}(\lambda_\alpha)
\]
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\[ \tau_n = |\text{Re}(\lambda_n)|^{-1} \]  
\hfill (7)

As a reference, hydraulic residence times needed to approach a steady state with a certain accuracy were calculated numerically by nonlinear model simulations.

2.3. Model-based Process Optimization

Optimal process inputs for dilution rate \( (D) \) and spent sulfite liquor concentration in the feed medium \( (c_{\text{SSL}}) \) were selected using a multi-objective optimization procedure. Biomass space time yield was defined as a typical objective to be maximized in bioprocess development, see Eq (8). In addition to that, time scale of the system, see Eq (9), was included as an additional objective function to obtain regions with high biomass productivity and fast equilibration kinetics. Time scales at operating points of interest were scaled by the maximum time scale of the process design space.

\[ \min_{D, c_{\text{SSL}}} J_1 = -c_{X, \text{steady state}} D \]  
\hfill (8)

\[ \min_{D, c_{\text{SSL}}} J_2 = \frac{\tau_{\text{max operating point}}}{\tau_{\text{max design space}}} \]  
\hfill (9)

Pareto-optimal solutions were computed using a genetic algorithm (gamultiobj, MATLAB R2017b) based on NSGA-II (Deb et al., 2002).

3. Results and Discussion

3.1. Influence of Process Parameters and Reaction Kinetics on Time Scale

Time scales were determined based on eigenvalue analysis of the linearized process model. Figure 1 shows the effect of two main operational parameters for CSTR processes, dilution rate and feed concentration, on the time scale of the analyzed process. Each Jacobian eigenvalue corresponds to the time scale of a mode, as depicted by dashed lines in Figure 1. The mode with the largest time scale at a certain operating point dominates the time scale of the process in the respective region, as can be seen by comparison to the numerically calculated residence times needed to approach a steady state in Figure 1 (solid line). When the dilution rate of the CSTR is approaching the washout of the system, in the region around 0.05 h\(^{-1}\) in Figure 1, time scale is considerably increasing. This is in accordance with the phenomenon of critical slowing down of nonlinear systems close to phase transitions (Jähnig and Richter, 1976; Lachmann and Schneider, 1983).

![Figure 1: Eigenvalue based time scales of modes (dashed lines) and numerically derived residence times (solid line) needed to approach steady state with \( \alpha = 0.05 \) in dependence of CTSR operational parameters dilution rate (A) and feed concentration (B).](image-url)
Figure 2: Eigenvalue based process time scale in dependence of CSTR operational parameters dilution rate and normalized feed concentration for different reaction systems. A: Multi-substrate process (spent sulfite liquor). B: Single carbon substrate process without inhibitory effects. The concentration of spent sulfite liquor in the feed influences the time scale of the process as well. Different substrate and inhibitor levels can shift the boundary of the CSTR washout equilibrium and thereby the region of slow time scales. In general, it can be stated that residence times significantly above the commonly applied three-residence-times rule of thumb are necessary to approach a steady state in most operational regions of the studied CSTR process. 10 residence times are sufficient for a wide range of the design space. However, close to the washout state up to 100 theoretical residence times are necessary to fully reach a steady state. Equilibration of the CSTR bioprocess is not governed by hydraulic equilibration but by the time scale of the biochemical reaction system. In Figure 2 A the process time scale is visualized for the entire process design space, depicting critical slowing down in the region close to washout. In comparison to that, a single substrate CSTR without inhibitory effects as described in Doran (2013) is shown in Figure 2 B. For this simpler reaction system, lower time scales and a less pronounced slowing down close to washout can be observed. The type of underlying reaction mechanisms, such as substrate competition, strongly influences the reaction velocity of a bioprocess, making time scale analysis a necessity to determine steady state stabilization timepoints during strain and bioprocess characterization.

3.2. Time Scale Analysis in Model-based Optimization

Time scale analysis can be included as an objective function within an optimization problem. By defining a multi-objective optimization problem, maximum space time yields for biomass productivity and minimal time scales for fast system responses were defined for the examined bioprocess. In Figure 3 A the process design space based on the sum of equally weighted objective function values is shown. In accordance to the critical slowing down of the system, undesirably high objective function values are obtained close to washout equilibrium. Washout regions are excluded from the process design space. To obtain optimal operating conditions among the conflicting objective functions for high biomass formation rates and fast system response, Pareto optimal solutions were computed by a genetic algorithm, see Figure 3 B. A time scale threshold below 80 h was set as constraint. Thereby, a dilution rate of 0.018 h\(^{-1}\) and a normalized spent sulfite liquor feed concentration of 0.51 were selected as optimal process parameters yielding a CSTR bioprocess with predicted 0.70 g L\(^{-1}\) h\(^{-1}\) biomass space time yield and a time scale of 68.8 h. This corresponds to 8.4 residence times needed to reach a steady state when simulating the nonlinear process.
3.3. Dynamic Response to Process Disturbances

The dynamic behavior during CSTR process start up as well as upon process disturbances was analyzed for the operating point optimized in section 3.2. Variability in lignocellulosic raw material characteristics is an important challenge for industrial biotechnology (Kenney et al., 2013). Therefore, the transitory behavior after disturbances in the substrate feed composition (25 %) is simulated for the optimized (dilution rate 0.018 h⁻¹, normalized feed concentration 0.51, productivity 0.70 g L⁻¹ h⁻¹) as well as for a time-scale suboptimal but equally productive operational point (dilution rate 0.018 h⁻¹, normalized feed concentration 0.84, productivity 0.71 g L⁻¹ h⁻¹). The startup phase until steady state and behavior after disturbance in the feed composition are displayed in Figure 4 A for the biomass and B for the substrate concentration. The optimized operational point (black line) reaches its initial steady state faster than the operational point with comparable productivity (grey line). Also, its response upon process disturbances and the subsequent return to its targeted operational point is considerably faster. This shows that time scale information can be used to define process conditions leading to a stable and productive process.
4. Conclusions

Time scale analysis delivered valuable insights into the dynamics of a wild-type *C. glutamicum* bioprocess utilizing lignocellulosic waste as a substrate. It could be shown that a high amount of residence times can be necessary to approach a steady state under certain operational parameters. This has implications for CSTR cultivations for strain characterization as well as industrial operations subject to process disturbances due to raw material fluctuations. Multi-objective optimization was performed to select operational points combining high productivity with fast steady state convergence. Time scale analysis is a straightforward, quantitative methodology and its inclusion within process design and development can help to correctly plan strain characterization experiments and to determine robust process conditions.

Acknowledgements

This project has received funding from the Bio Based Industries Joint Undertaking under the European Union’s Horizon 2020 research and innovation program under grant agreement No 790507 (iFermenter).

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