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# Optimization of Operating Conditions for Ferrichrome Production in a Membrane Bioreactor Using *Ustilago maydis*

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## Abstract

In this work, a continuous siderophore production system using the phytopathogenic fungus *Ustilago maydis* is considered. A hybrid process, specifically, a microfiltration membrane bioreactor is employed which is deemed to be advantageous since cells are retained in the vessel while possibly inhibitory products are continuously withdrawn from the system. Accordingly, the process is operated at high cell density thereby increasing productivity. Preliminary analysis and studies for steady state optimization result in the existence of steady-state points for the maximum production rate. Thus, the optimization focus is divided into two operation stages: the startup and the steady-state operation. The former operation implies a dynamic optimization where the optimal policies are determined in order to meet the previously established optimal steady-state. Furthermore, the startup period is characterized through a switching operation mode from batch to continuous. However, the overall aim of the optimization is the maximization of the product total amount per time while minimizing the startup period. To keep the production costs at a convenient level, different constraints are included in the optimization problem such as a glucose waste limit, a lower bound for the outlet product concentration, and also technical constraints which involve upper bounds for the biomass concentration in the reactor. For the simulation and computation of the sensitivities, we propose a new multiple-time-scaling-approach to solve the resulting optimization problem which possesses strong nonlinear properties.

**Keywords**: *Ustilago maydis*, membrane bioreactor, high cell density, model-based optimization.

## 1. Introduction

Ferrichromes belong to a group of iron chelating agents called siderophores. Possible clinical applications of these compounds include elevating the potency of antibiotics, and anti-cancer drugs. *Ustilago maydis*, a host specific phytopathogenic fungus (smut fungus), can be used for the production of ferrichrome. In liquid culture, *U. maydis* grows yeast-like in oval shaped single cells (length approx. 10μm, see Fig. 1).
A membrane bioreactor (MBR) is defined as a hybrid process since it combines a common bioreactor and membrane separation units for biomass retention offering process intensification and new possibilities for bioprocesses and wastewater treatment.

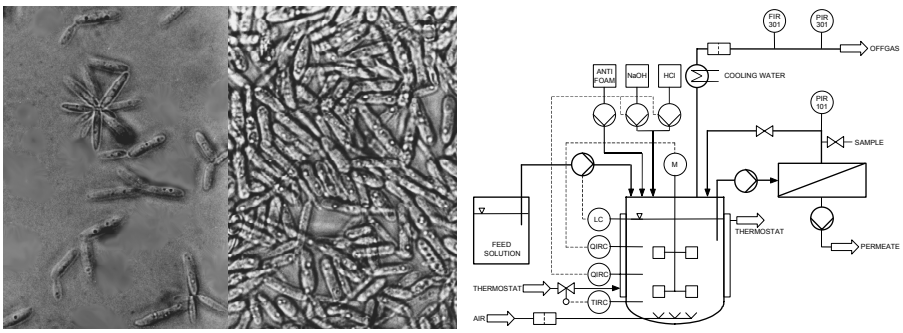



Figure 1. a) *U. maydis* after one and three days of cultivation; b) experimental setup.

MBR combine the benefits of high biomass concentrations to increase volumetric productivity with the possibility to run a continuous process at controlled biomass retention and thereby controlled biomass growth rate. With the organism used in this work, ferrichrome productivity was increased by a factor of 12 in comparison to repeated batches (Drews and Kraume, 2005). By decoupling of hydraulic and biomass retention times, MBRs offer an additional degree of freedom for process control in comparison to chemostats. Therefore, they can be particularly advantageous in fermentations where inhibitory metabolites occur either as the desired product or as unwanted byproducts since the productive cells are retained in the vessel while soluble products are continuously withdrawn. Although several practical experiences and data are already available for design and operation of MBR processes there is still considerable optimization potential. Here, e.g., to optimize the productivity in MBR, optimization under product inhibition and process constraints is required. Product inhibition means that the product concentration has a strong impact on the growth rate with a decreasing effect at either too high or too low concentrations and an optimum somewhere in between. Thus, the potential for optimization is mainly caused by this inhibitory effect. However, due to process constraints, mostly derived from technical and cost restrictions, it is not always possible to keep the concentrations of the product and other inhibitory components exactly at their optimum level. Consequently the optimization potential is mainly exploited by finding an operation policy, where the compliance of those process constraints is always assured while keeping the concentrations of the inhibitory components as close, as soon and long as possible to the optimum productivity level over the entire time horizon. This makes model-based optimization strategies a challenging task.

## 2. Experimental Procedure

*Ustilago maydis* (strains *urbs*- and wild type FB1) was stored in glycerol stocks (25 %) at –80 °C. After a 3-day inoculation on potato-dextrose-agar cells were transferred into shaking flasks containing a modified Grimm-Allen-medium with glucose as the main carbon source. Flasks were shaken for approx. 24 h at 100 $min^{-1}$ and at 27 °C until they were transferred into a 5 L glass fermentor. In MBR runs, this was equipped with an external ceramic tubular membrane module for biomass retention. Temperature was controlled at 27 °C, pH at 7.2, and $pO_2$ at 40 %. Fig. 1b shows the experimental setup. To study the effects of glucose and ammonia limitation, respectively, and the effects of short- and long-term limitation, different batch, fed-batch, chemostat and MBR cultivations were carried out. The biomass concentration was determined by turbidity

measurements at 600 nm calibrated against dry weight measurements of washed cells. For determination of substrates and nutrients concentrations, commercial test kits were used. The concentration of ferrichromes in the supernatant was measured by the CAS-method (Schwyn and Neilands, 1987) whereby unchelated siderophores can be detected. All samples were membrane filtered before analyses.

## 3. Modeling of MBR

A model was developed to describe the considered MBR process. The model approach includes mass balances and kinetics. At the given conditions (pH 7.2 and T = 27 °C), the concentrations of glucose $c_C$, ammonia $c_N$ and ferrichrome $c_P$ were identified as the main influencing parameters for growth of *Ustilago maydis*. Thus, the following expression is derived. For glucose, a simple Monod term was chosen while for ammonia and ferrichrome inhibition was observed at higher concentrations:

$$\mu = \mu_{\max} \cdot \frac{c_C}{c_C + K_{S,C}} \cdot \frac{c_N}{c_N + K_{S,N} + \frac{c_N^2}{K_{I,N}}} \cdot \frac{c_P}{c_P + K_{S,P} + \frac{c_P^2}{K_{I,P}}} \tag{1}$$

with $\mu_{max} = 0.28\ h^{-1}$, $K_{S,C} = 0.7\ gL^{-1}$, $K_{S,N} = 2 \cdot 10^{-4}\ gL^{-1}$, $K_{S,P} = 6 \cdot 10^{-3}\ gL^{-1}$, $K_{I,N} = 1.95\ gL^{-1}$, and $K_{I,P} = 0.115\ gL^{-1}$. The kinetic parameters specific growth, substrate uptake and production rates depend on different conditions such as substrate and production concentrations, among others. Balance equations for the individual components (biomass, substrates, nutrients, and metabolites) are coupled via yield coefficients *Y*. These are defined as the rate of change in one concentration over the rate of change in another. Especially for steady states, i.e., low growth rates, maintenance concepts need to be implemented where substrate uptake only yields energy for cell survival. The following set of equations describes the system:

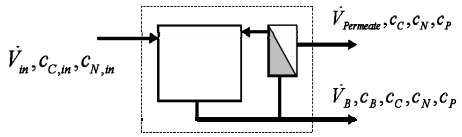
$$\frac{dV_R}{dt} = 0 = \dot{V}_{in} - \dot{V}_{Permeate} - \dot{V}_B \tag{2}$$

$$V_R \cdot \frac{dc_B}{dt} = -\dot{V}_B \cdot c_B + \dot{r}_B \cdot V_R \tag{3}$$

$$V_R \cdot \frac{dc_C}{dt} = \dot{V}_{in} \cdot \left(c_{C,in} - c_C\right) + \dot{r}_S \cdot V_R \tag{4}$$

$$V_R \cdot \frac{dc_N}{dt} = \dot{V}_{in} \cdot \left(c_{N,in} - c_N\right) + \dot{r}_N \cdot V_R \tag{5}$$

$$V_R \cdot \frac{dc_P}{dt} = -\dot{V}_{in} \cdot c_P + \dot{r}_P \cdot V_R \tag{6}$$




$$-\dot{r}_S = \frac{\dot{r}_B}{Y^g_{B/S}} + k_{m,S} \cdot c_B \tag{7}$$ substrate uptake

$$-\dot{r}_N = Y_{N/B} \cdot \dot{r}_B \tag{8}$$ ammonia uptake

$$\dot{r}_P = Y^g_{P/B} \cdot \dot{r}_B + k_P \cdot c_B \tag{9}$$ production rate

For each time point, the degree of freedom is four. In case of open loop control, it is recommendable to use the trajectories of the input variables $\dot{V}_{in}$, $\dot{V}_B$, $c_{C,in}$ and $c_{N,in}$ as control or decision variables in the optimization task. For the dynamic optimization, in particular, the initial concentrations of all components, particularly the substrates, may be used as additional decision variables.

## 4. Steady state and dynamic optimization

*4.1. Formulation of the optimization problems*

The general aim of the optimization is the optimal increase of the ferrichrome productivity. Thus, the objective function is defined as the maximization of the total amount of produced ferrichrome divided by the time. The equality constraints are the model equations. Some economic and constructional aspects give rise to further constraints. Since there is an upper bound for the flux through the membrane, and for the membrane area to be coupled to a given reactor volume, the hydraulic residence time $\tau$ is restricted (liquid hold-up in the membrane module < reactor volume). With a typical value of 20 L $m^{-2}h^{-1}$ for filtration of biological suspensions and a typical tubular module packing density of 450 $m^2m^{-3}$, the minimum residence time $\tau_{min}$ is estimated to be 0.11 h. Furthermore, through increased viscosity and uptake rates, high cell densities result in decreasing oxygen transfer rates and thereby increased operating costs. A value of 200 $gL^{-1}$ is chosen as the maximum allowable $c_B$ (dry weight). Overall operating costs are also influenced by raw material utilization and product purification. In order not to waste an excess of raw materials, the maximum allowable $c_C$ is also restricted. Additionally, an upper bound for $c_N$ of 1.5 $gL^{-1}$ is required to avoid poisoning of the biomass. Moreover, because too low product concentrations cause difficulties and excessive operating costs in product recovery in downstream separation units, e.g., adsorption processes, a lower bound for $c_P$ is considered.

It should be noted, that the bounds for glucose and ferrichrome are only valid when there is an outflow, consequently there must be an inflow. In batch periods with no in- and outflow, a violation of those bounds is feasible. In contrast, the bounds of $c_N$ and $c_B$ should never be violated (hard constraints). Concerning the bounds of the decision variables, the upper bound of the volumetric inflow rate is determined by $\tau_{min}$. The lower bounds of all decision variables can be set to zero.

The formulation of the optimization problem above corresponds to the dynamic optimization. For the steady state optimization, the objective function will be simplified to the maximization of the ferrichrome production rate, and for the constraints, all time dependent variables such as the derivatives of the state variables will be omitted.

*4.2. Solution Strategy*

The steady state problem is relatively small and is solved by the simultaneous approach. Contrary to that, for the dynamic optimization problem, trajectories of the decision variables within a large time horizon need to be computed which requires a discretisation of the time horizon into a large number of time intervals leading to a large scale optimization problem. In this work, the sequential approach is applied. Due to the rather expected non-monotonic behavior of state variables within time intervals (i.e. maxima of glucose and ammonium, and minima of product concentrations) where the feed parameters are constant, the orthogonal collocation method in finite elements (5-points) is used as a discretization approach to guarantee robustness and efficiency at each simulation step (Arellano-Garcia et al., 2005). In addition, a step-size control is integrated to assure convergence at each time interval.

*4.2.1. Decomposition approach*

An inherent characteristic of the MBR process is that a batch phase is required at the beginning when the initial condition of the inhibitory product is very small, i.e. far from its peak which is optimal for the growth of biomass. Furthermore, since the volumetric flow rates are set to zero during the batch period, all the other continuous decision variables have no impact on the process either. On the other hand, the initial

concentrations of the substrates, and the switching time point from the batch to the continuous phase are crucial decision parameters for the objective function and the constraints in the optimization problem. Thus, the original optimization problem is decomposed into two sub-problems. The first one minimizes the batch time subject to end conditions concerning concentrations bounds, which are supplied by the optimizer of the continuous phase where initial concentrations are used as decision parameters as well. This interaction procedure is finished when the end concentrations of the batch period are equal to the corresponding optimized values of the continuous period. For MBR, however, the reformulation of the subproblems is physically equivalent to the original problem formulation.

*4.2.2. An approach to multiple time-scaling*

Due to the large time horizon of the MBR process, the trajectories of the decision variables are discretized into time intervals where the values are piecewise constant. It should be noted, that the size of these time intervals is independent of those used for simulation. The constraints are to be met at all time points in certain or even all periods. However, to solve the optimization problem in the continuous phase, a step size control algorithm is required in the simulation stage to assure robust convergence. Furthermore, it must be guaranteed that a collocation point of the larger time interval, which is used for the decision variables and constraint calculation, is matched with the end of a small time interval. By this means, an accurate sensitivity calculation can be performed which is required for the NLP solver in the optimization layer. This leads to a novel approach of multiple time-scaling where the step size control (inside one large time interval) is carried out by a 3-layer system (Fig. 2). At the beginning of computing the trajectories, the step length is first set up in Layer 1, where it is equal to the time interval for the decision variables. In case of convergence, the sensitivities concerning the constraint values at all collocation points are calculated simultaneously. If not, the step size control goes one layer down and so on forth. In Fig. 2, DT or DTs represents the general term for the interval length while the subscripts u and c indicate the correspondence either to the decision interval (ii) or to the interval between the current collocation points, respectively. However, it can also be seen that the end of one interval in Layer 3 is always limited to the next collocation point.

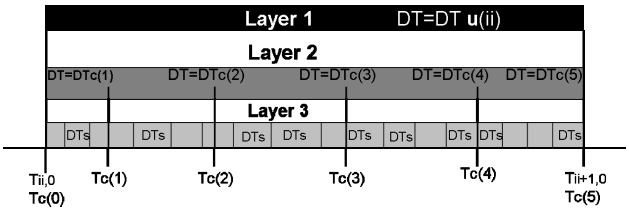



Figure 2. Layer system for the multiple time scaling

## 5. Computation results

The numerical results begin with preliminary steady state studies where the upper bound of glucose and biomass is considered. For this purpose, steady state optimization is carried out with different upper bounds for glucose concentration over different values of the residence time. These results allow two main conclusions: *i)* only when the glucose concentration is lower than approx. $5gL^{-1}$, significant differences can be observed due to the fact that $K_{S,C} = 0.7\ gL^{-1}$; *ii)* if the residence time is lower than 2 hours, a major impact on optimal production and growth rate is identified. Due to the

fact that $c_B$ is always at its upper bound and $c_N$ at its optimal value, the optimal value of $c_P$ can actually be described as a function of $c_C$ and $\tau$. Apart from $c_P$, the dependent state variables remain at a level for maximum allowable $\mu$ and, thus, the optimal steady state production rate can never be exceeded during the dynamics of the entire process. Therefore, the dynamic optimization problem is equivalent to a startup problem.

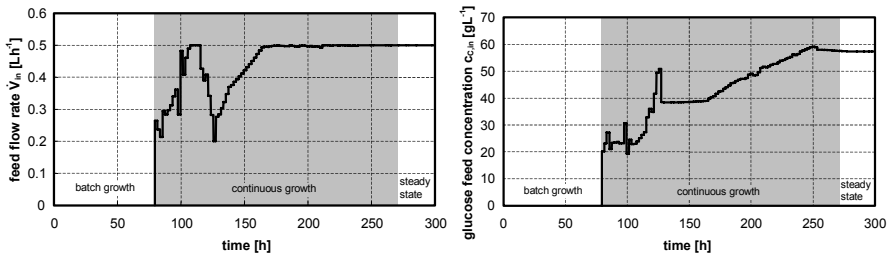



Figure 3. Optimal trajectories of the volumetric feed rate and of the glucose feed concentration.

Due to the fact that the maximum possible $c_B$ results from the steady state optimization, and that the growth rate is only limited by the constraints of $c_C$ and $\tau$, it is obvious, that the production rate has reached its maximum value at the steady state point. Thus, the entire optimal operation strategy can be roughly divided into three periods: batch growth, continuous growth and steady state period. The most significant trajectories of the decision variables are illustrated in Fig. 3. The results show that the feed flow rate rapidly increases at the beginning of the continuous growth period until $\tau_{min}$ is reached in order to keep $c_P$ as long as possible at its lowest possible point, which is the closest point to its peak in the growth rate function. From that state on, the feed flow rate keeps close to its end value in the steady state period, while the feed concentrations of the substrates approach to their steady state end values relatively continuously in order to compensate the increasing consumption by the growing biomass.

## 6. Concluding remarks

In this work, model-based optimization is applied for ferrichrome production in an MBR using *Ustilago maydis*. A model is used which was validated with experimental data. For the batch period, instead of the feed conditions, the initial conditions of the feeding substrates and the time point to switch to the continuous period are critical parameters for optimality. Thus, a novel developed decomposition method has been applied for matching the batch period with the following continuous growth period. For the numerical optimization of the growth period, it is necessary to assure robustness in convergence and a reliable sensitivity calculation for the constrained variables at different selected time points. For this purpose, a novel efficient approach to multiple time scaling strategy is proposed.

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