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Model-based State Estimation Based on Hybrid Cybernetic Models

L. Carius [*] **J. Pohlodek** [*] **B. Morabito** [*,***] **A. Franz** [*]
M. Mangold [****] **R. Findeisen** [*,***] **A. Kienle** [*,**]

[*] *Institute for Automation Engineering, Otto-von-Guericke-University Magdeburg, Germany*
[**] *Process Synthesis and Dynamics Group, Max Planck Institute for Dynamics of Complex Technical Systems, Magdeburg, Germany*
[***] *International Max Planck Research School (IMPRS) Magdeburg, Germany*
[****] *Engineering Mathematics, University of Applied Science Bingen, Germany*

Abstract: Biotechnological processes still represent a challenge for process optimization and automation as the data landscape consists of unavailable, inaccurate, delayed or missing measurement information. As a first step towards automation of biotechnological processes, methods have to be refined for estimating the unknown states with an acceptable precision, using a mathematical model of the system. Due to the technological advances, knowledge and computational powers are constantly increasing so that models of a higher complexity and predictive quality are now available. Hybrid cybernetic models offer a flexible, yet detailed description of the biotechnological process under consideration. They connect the nonlinear system dynamics to the metabolic information of the organism and allow to consider cell internal regulations. In this work we explore if this class of models can be successfully applied for real-time process monitoring. We do this by evaluating the performance of two commonly used state estimators, an unscented Kalman filter and a moving horizon estimator, which both use a hybrid cybernetic model to observe the non-linear process of poly-β-hydroxybutyrate production in the organism *Cupriavidus necator*. To our knowledge this is the first time that this class of models is used for model-based process observation.

Keywords: model-based, non-linear state estimation, process monitoring, hybrid cybernetic modeling, industrial application, poly-β-hydroxybutyrate production, PHB

1. INTRODUCTION

Monitoring and control of biotechnological processes requires the knowledge of the states of the system. In spite of the recent progress in the development of technologies for the non-invasive process analysis (Hinz (2006)), the measurement information which can be obtained during an industrial production process remains limited. If the systems states cannot be measured directly, or if the measurements contain uncertainties, they may be obtained from measurement information via state observers. For estimating these unknown states, the observer requires a model of the process dynamics, inputs and measurements of the system. In this work we consider a hybrid cybernetic model (HCM) to describe and observe the process behaviour of the biological system. The HCM modelling approach is of special interest, as it allows a detailed description of the system, taking into account, the relevant metabolic pathways of the organism in form of a reduced set of elementary modes, the dynamics of the process in form of ODEs, and information about cellular regulation, through the introduction of cybernetic variables. In other words, the dynamics of all external states of the system with all the regulated reaction rates, are combined with a linear algebraic equation system, which describes the stoichiometry of the relevant metabolic pathways, and thus, the intracellular fluxes of the system (Kim et al. (2008); Song and Ramkrishna (2009)). The introduction of the cybernetic variables allow the system to switch between different growth scenarios. According to a work of Song et al. (2009), HCMs yield the highest descriptive quality when compared to other commonly used metabolic modeling approaches, namely macroscopic bioreaction models and dynamic flux balance analysis. It is the main contribution of this paper to investigate if the unscented Kalman filter (UKF, Julier and Uhlmann (1997)) and the moving horizon estimator (MHE) (Rawlings (2013) and references therein) can be applied successfully for monitoring a nonlinear biotechnological process which is represented by a HCM. The UKF belongs to a family of estimation approaches derived from the well known Kalman filter (Kalman (1960)). These are based on a probabilistic interpretation of the estimation problem. The unknown states are described through probability distributions. Starting from a guessed initial distribution, Kalman filters operate in a recursive way. The new estimate of the states is determined by propagating the previous states through the system and using the measurements at the current time

step to update the probability distribution. Past measurements are approximated and summarized in the covariance matrix and in the estimated states of the previous time step. Generally for nonlinear systems even if the initial distribution is Gaussian, it does not remain Gaussian at future times. Describing such distributions can be computationally demanding, therefore several approximations have been proposed. One way to approximate the distribution evolution is by using the UKF approach. This method has been shown to describe accurately the future mean and covariance up to the third order of a Taylor series expansion (Julier (2002)). The MHE estimates the states by minimizing the mismatch between the real measurements and the model measurements along a shifting time horizon. Within this horizon no information is lost since no approximations are made. Due to computational time limitations, the horizon has a finite length. The information left behind the horizon is carried on by a so called *arrival cost* which summarizes past information in one single term. If the system is non linear this term only approximates the past information. Notice that despite this approximation is similar to the approximation introduced in the UKF, it is less critical since no loss of information occurs within the time horizon. The MHE approach can be seen from a purely deterministic prospective. In this method no considerations of the probability distribution of the system variables is necessary, although a connection with the probabilistic prospective exists and it can be easily shown in simple cases (Rawlings and Mayne (2009)). Furthermore, in some cases probabilistic considerations can be useful for weights selection. In this contribution the performance of both observers is evaluated considering a HCM which describes the poly-β-hydroxybutyrate (PHB) production in the organism *Cupriavidus necator* (Franz et al. (2011)).

2. MODEL

The HCM is based on a metabolic network for the production of PHB in *Cupriavidus necator* alias *Ralstonia eutropha*, which has 36 reactions (see Figure 1) and uses the external metabolites fructose (carbon source) and ammonium chloride (nitrogen source) to control PHB production (Franz (2015); Franz et al. (2011)). In the given growth scenario, PHB production is initiated if the carbon source is supplied in excess, whilst the nitrogen source limits the growth. To allow online estimation of the product, a measurement equation reconstructing the intracellular PHB content from the absorbance measurement of the culture broth is implemented.

2.1 State Equations

If the stoichiometric coefficients of the metabolic network are condensed in the stoichiometric matrices N_i, where $i \in \{\mathrm{ex}, \mathrm{in}, \mu\}$ with the dimension $n_i \times n_r$, and if the reaction rates are condensed in vector $r(t)$, the temporal change of the concentration of the external metabolites c_{ex} in [g/l] and internal metabolites c_{in} in [g/g biomass], and the change of the biomass concentration c_{bio} in [g/l] can be written in matrix notation (Heinrich and Schuster (2012); Kim et al. (2008))

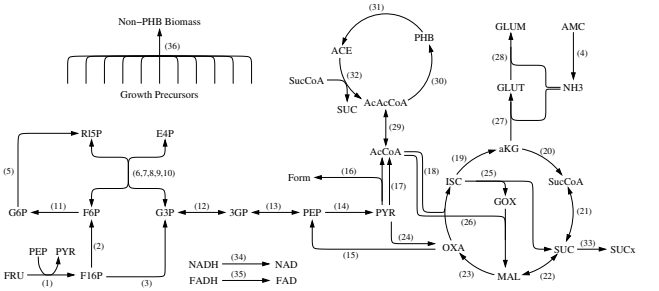

Fig. 1. Used metabolic network for *Ralstonia eutropha*.

$$\dot{c}_{\mathrm{ex}}(t) = N_{\mathrm{ex}} r(t) c_{\mathrm{bio}}, \tag{1}$$
$$\dot{c}_{\mathrm{in}}(t) = N_{\mathrm{in}} r(t) - N_{\mu} r(t) c_{\mathrm{in}}, \tag{2}$$
$$\dot{c}_{\mathrm{bio}}(t) = N_{\mu} r(t) c_{\mathrm{bio}}. \tag{3}$$

It is a general assumption of metabolic modeling approaches, that intracellular reaction rates are very fast, compared to the extracellular reaction rates, and thus all internal metabolites are in quasi-steady state (Stephanopoulos et al. (1998)). However, as a storage compound with slow dynamics the intracellular metabolite PHB has to be excluded from this assumption. Therefore, the system of differential equations is extended by

$$\dot{c}_{\mathrm{PHB}}(t) = N_{\mathrm{PHB}} r(t) - N_{\mu} r(t) c_{\mathrm{PHB}}. \tag{4}$$

The derivates of the differential equation (2) describing the remaining intracellular metabolites can be set to zero

$$\dot{c}_{\mathrm{in}}(t) = 0 = N_{\mathrm{in}} r(t) - N_{\mu} r(t) c_{\mathrm{in}}. \tag{5}$$

Further it is assumed, that the dilution of internal metabolites c_{in} caused by the growth of the biomass is much slower than internal reaction rates, $N_{\mu} r(t) c_{\mathrm{in}} = 0$ follows. Now the intracellular fluxes are represented by a homogenous system of linear equations

$$N_{\mathrm{in}} r(t) = 0, \tag{6}$$

and Equation (2) is excluded from the set of ODEs. The solutions of Equation (6) are the steady-state rate distributions, referred to as flux modes in literature, and span the flux space of the whole network.

2.2 HCM

Decomposition of the metabolic network A first step of HCM is the decomposition of the metabolic network into a set of active flux modes, referred to as relevant elementary modes (EM), which are required for describing the metabolic behavior under the given growth conditions (Kim et al. (2008); Song and Ramkrishna (2009)). In case of our model out of 122 EMs, 3 active EMs were selected and shown to be in good agreement with experimental data (Franz (2015)). The HCM approach further assumes that every relevant EM is catalyzed by one key enzyme. Level and activity of these enzymes are incorporated in the flux vector r_{M}, which represents the fluxes trough the EMs. The vector of the reaction rates $r(t)$ is then expressed by the elementary mode decomposition Z ($n_r \times n_{\mathrm{EM}}$, where n_{EM} is the number of EMs) and the vector of reaction rates (or fluxes) r_{M} through the EMs

$$r(t) = Z r_{\mathrm{M}}(t). \tag{7}$$

When this extension is included into the model, the existing ODEs of the system can be rewritten to

$$\dot{c}_{\mathrm{ex}}(t) = N_{\mathrm{ex}} Z r_{\mathrm{M}}(t) c_{\mathrm{bio}}, \tag{8}$$
$$\dot{c}_{\mathrm{PHB}}(t) = N_{\mathrm{PHB}} Z r_{\mathrm{M}}(t) - N_{\mu} Z r_{\mathrm{M}}(t) c_{\mathrm{PHB}}, \tag{9}$$
$$\dot{c}_{\mathrm{bio}}(t) = N_{\mu} Z r_{\mathrm{M}}(t) c_{\mathrm{bio}}, \tag{10}$$

and an additional ODE describing the dynamics of the enzyme levels of the key enzymes which catalyze the relevant EMs

$$\dot{c}_{e,i}(t) = \delta_i + r_{\mathrm{EM},i} b - \varepsilon_i c_{e,i} - \mu c_{e,i}, \tag{11}$$

have to be introduced. $c_{e,i}$ represent the nondimensional enzyme levels of the key enzymes for the i-th EM, $r_{\mathrm{EM},i}$ the synthesis rates of these key enzymes and $b = 1 - c_{\mathrm{PHB}}$ is the catalytical active part of the biomass. The parameters δ_i represent the constitutive enzyme synthesis rate constants and ε_i the enzyme degredation constants.

Introducing the cybernetic control variables As the last step of HCM derivation, the internal regulation of the cell is considered by introducing the cybernetic control variables u and v which control the synthesis and activity of the relevant key enzymes. v_i controls the reaction rates through the EMs

$$r_{\mathrm{M},i} = v_i c_{e,i,\mathrm{rel}} k_{r,i} \underbrace{\prod_{j \in L(i)} \left(\frac{c_{s,j}}{K_{i,j} + c_{s,j}} \right)}_{\rho_i}, \tag{12}$$

and, u_i controls the enzyme synthesis rates

$$r_{\mathrm{EM},i} = u_i k_{e,i} \rho_i, \tag{13}$$

where $c_{e,i,\mathrm{rel}}$ are the relative enzyme levels, $k_{r,i}\rho_i$ and $k_{e,i}\rho_i$ describe Monod type kinetics (Monod (1949)), and $L(i)$ is the set of metabolites associated to the i-th EM. According to Franz et al. (2011) it is a robust assumption, that the enzyme levels are quasi-stationary. Thus, Equation (11) is excluded from the set of ODEs and the relative enzyme levels can be approximated

$$\dot{c}_{e,i}(t) = 0 \quad \rightarrow \quad c_{e,i,\mathrm{rel}} \approx u_i b. \tag{14}$$

For detailed description of this approximation, see Franz et al. (2011). It follows, that in the reduced version of the HCM the enzyme synthesis rates $r_{\mathrm{EM},i}$ are neglected. The cybernetic variables u_i and v_i are now both controlling the fluxes through the EMs

$$r_{\mathrm{M},i} = v_i u_i b k_{r,i} \rho_i. \tag{15}$$

The control laws that define the cybernetic variable are based on the assumption, that the cell is optimally regulated and, thus, a certain objective is maximized

$$u_i = \frac{f_i \rho_i}{\sum f_i \rho_i}, \tag{16}$$
$$v_i = \frac{f_i \rho_i}{\max f_i \rho_i}, \tag{17}$$

where $f_i\rho_i$ is the return of investment, which can be calculated from the metabolic objective function. Here, the objective function is to maximize the uptaken carbon. Hence, the vector of the weighting factor f contains the normalized number of uptaken carbon units. Due to the introduction of these cybernetic variables, the HCM representation of the system allows the process to switch between different growth phases, as u_i and v_i distribute the substrate uptake flux among the three EMs of the metabolic network in order to represent all the phases of a process adequately (Song et al. (2009)). This is in important feature for the description of the PHB production process, since, in dependence of the environmental and cell internal conditions, the organism can switch between cell growth, synthesis of PHB and metabolization of PHB.

2.3 Measurement Equation

In this work a measurement equation, which reconstructs the PHB concentration (c^m_{PHB}=$c_{\mathrm{PHB}} c_{\mathrm{bio}}$ in [g/l]) from the absorbance measurement (A) of the culture broth at a wavelength of $\lambda = 600\,\mathrm{nm}$ is used

$$A(\lambda) = \underbrace{\varepsilon_{\mathrm{PHB}}(\lambda) l}_{k_{\mathrm{PHB}}(\lambda)} c^m_{\mathrm{PHB}} + \underbrace{\varepsilon_{\mathrm{res}}(\lambda) l}_{k_{\mathrm{res}}(\lambda)} c_{\mathrm{res}}, \tag{18}$$
$$c_{\mathrm{bio}} = c_{\mathrm{PHB}} + c_{\mathrm{res}}. \tag{19}$$

Here, total biomass is viewed as a combination of two compartments, namely the stored PHB and the residual biomass (c_{res} in [g/l]). This equation is based on the assumption that A reflects changes in the distribution of the cell size, and, that an increase of the cell size can be attributed to high PHB content of the cell (Franz (2015)). Using data of 4 batch cultivations Franz (2015) determined the values of both constants k_{PHB} and k_{res} for each experiment.

3. STATE ESTIMATION

The goal is using UKF and MHE observers for estimating the states of the system, i.e. concentrations of PHB, the total biomass, and the substrates fructose and ammonium chloride using the absorbance measurement. They rely on the system model and measurement model reported in Section 2. These models can be described in a more general form as follows

$$\dot{x}(t) = f(x(t)) + \zeta, \tag{20}$$
$$y_k = h(x_k, p) + \eta_k. \tag{21}$$

Here, $x \in \mathbb{R}^{n_x}$ denotes the state vector, $y \in \mathbb{R}^{n_y}$ denotes the measurement vector and $p \in \mathbb{R}^{n_p}$ the parameter vector. The process noise ζ and the measurement noise η_k are assumed to be zero mean normally distributed with the covariance matrices Q and R, respectively. Note that the measurement equation is in discrete time while the dynamic equation is in continuous time. The symbol $(\hat{\cdot})$ is used to indicate the estimated variables.

3.1 Unscented Kalman Filter

Here, the main idea of the UKF is shortly presented. For more details the reader is referred to Wan and Van Der Merwe (2000). The algorithm starts by choosing a number of points, called *sigma points*, which represent the initial probability distribution. Different sigma points selection criteria have been proposed (Julier et al. (1995); Julier and Uhlmann (1997); Julier et al. (2000)). In this paper they are chosen such that they match the first two moments of the prior distribution, namely mean and covariance

$$\mathcal{X}_{k-1} = \left[\hat{x}_{k-1}, \hat{x}_{k-1} + \gamma \sqrt{P_{k-1}}, \hat{x}_{k-1} - \gamma \sqrt{P_{k-1}} \right]. \tag{22}$$

The parameters $\gamma = \sqrt{n_x + \lambda}$ and $\lambda = \alpha^2 (n_x + \kappa) - n_x$ are scaling parameters where $\alpha \in (0, 1]$ determines the spread of the sigma points and $\kappa \geq 0$ is a secondary

scaling parameter. These sigma points are then propagated through the system dynamics $\dot{\mathcal{X}}(t) = f(\mathcal{X})$ with the initial condition $\mathcal{X}(t_{k-1}) = \mathcal{X}_{k-1}$. The resulting propagated sigma points are indicated as $\mathcal{X}_k^-$. The predicted states and covariance matrix are computed as

$$\hat{x}_k^- = \sum_{j=0}^{2n_x} w_j^{(m)} \mathcal{X}_{j,k}^-, \tag{23}$$

$$P_k^- = \sum_{j=0}^{2n_x} w_j^{(c)} (\mathcal{X}_{j,k}^- - \hat{x}_k^-)(\mathcal{X}_{j,k}^- - \hat{x}_k^-)^\mathsf{T} + Q, \tag{24}$$

where $w_j^{(m)}$ and $w_j^{(c)}$ are given weights. The next step is to calculate the solution of the measurement equation for each sigma point $\mathcal{Y}_k = h(\mathcal{X}_k^-)$ and to approximate the predicted measurement, the corresponding covariance matrix and the cross-covariance matrix

$$\hat{y}_k = \sum_{j=0}^{2n_x} w_j^{(m)} \mathcal{Y}_{j,k}, \tag{25}$$

$$P_{y_k,y_k} = \sum_{j=0}^{2n_x} w_j^{(c)} (\mathcal{Y}_{j,k} - \hat{y}_k)(\mathcal{Y}_{j,k} - \hat{y}_k)^\mathsf{T} + R, \tag{26}$$

$$P_{x_k,y_k} = \sum_{j=0}^{2n_x} w_j^{(c)} (\mathcal{X}_{j,k}^- - \hat{x}_k^-)(\mathcal{Y}_{j,k} - \hat{y}_k)^\mathsf{T}. \tag{27}$$

The final step is to update the prediction with the current measurement

$$K_k = P_{x_k,y_k} \left(P_{y_k,y_k}\right)^{-1}, \tag{28}$$

$$\hat{x}_k = \hat{x}_k^- + K_k(y_k - \hat{y}_k), \tag{29}$$

$$P_k = P_k^- - K_k P_{y_k,y_k} K_k^\mathsf{T}. \tag{30}$$

To make the estimation more robust, the UKF was implemented in square root form (van der Merwe and Wan (2001)). Extension to this filter for parameter estimation can be done simply by considering the parameters as states with zero dynamics, namely $\mathrm{d}p/\mathrm{d}t = 0$.

3.2 Moving Horizon Estimation

The MHE is an optimization-based estimation technique which is based on the minimization of the error between the measured output and the estimated output. The minimization considers measurement and state noise within a time window, called *horizon*, in order to estimate the current state. Once this state has been estimated, the horizon slides one step ahead, takes a new measurement, and the minimization is repeated. For further details please refer to Robertson et al. (1996); Rao et al. (2003); Rawlings and Mayne (2009). The state estimate at time step k is given by solving, in general, a *nonlinear programming problem*

$$\min_{\hat{x}_{k-N|k},\hat{\zeta},\hat{p}} \left\{ \mathcal{L}(\hat{x}_{k-N|k}, \hat{\zeta}, \hat{p}) \right\}, \tag{31}$$

subject to

$$e(\hat{x}_{i|k}) = 0 \quad i = [k-N, ..., k], \tag{32}$$

$$d(\hat{x}_{i|k}) \leq 0 \quad i = [k-N, ..., k]. \tag{33}$$

The notation $(\cdot)_{i|j}$ refers to the variable $(\cdot)$ at time i estimated at time j, N is the horizon length, $\hat{\zeta}$ is the vector on state noise $[\hat{\zeta}_{k-N|k}, ..., \hat{\zeta}_{k|k}]$. $e(\cdot)$ and $d(\cdot)$ represent respectively equality and inequality constraints. The state dynamics will be considered in the equality constraints. Furthermore the concentrations are constrained to be positive by the inequality constraints. In this work, the following quadratic objective function is minimized

$$\mathcal{L}(\hat{x}_{k-N|k}, \hat{\zeta}, \hat{p}) = \left\| \begin{matrix} \hat{x}_{k-N|k} - x^*_{k-N|k} \\ \hat{p} - p^* \end{matrix} \right\|^2_{\mathcal{P}_{k-N|k}} + \\ + \sum_{i=k-N}^{k} \|\hat{\zeta}_i\|^2_{\mathcal{Q}} + \sum_{i=k-N}^{k} \|\hat{y}_i - \tilde{y}_i\|^2_{\mathcal{R}}. \tag{34}$$

The notation $\|x\|_A^2$ stands for the weighted vector norm $x^T Ax$. The matrices $\mathcal{P}_{k-N|k}$,$\mathcal{Q}$ and $\mathcal{R}$ are weights that can be tuned by the user. The state vector $x^*_{k-N|k}$ is the best estimate available of time $k-N$ and parameter vector p^* are the best current parameter estimate. The first term of the sum has the role to summarize the information the MHE leaves out of the horizon. The matrix $\mathcal{P}_{k-N|k}$ can be updated at every time step using a covariance update algorithm, for example the covariance update of Section 3.1 (Rawlings and Mayne (2009)).

4. RESULTS AND DISCUSSION

Here the performance of the UKF and the MHE for monitoring the concentration of PHB from the measurement information is studied. Considering the system equations of the model (Eq. (8)-(10), (15)-(17)) and the parameters derived by Franz (2015), the observer estimates the states $\hat{x}_k$ at each time step k, while the measurement of the absorbance $y_k = A_k$ is taken as the input. For simulating the plant and generating the measurement data, parameters for the HCM (Eq.(8)-(10), (15)-(17)) and the measurement equation (Eq.(18), $k_{\mathrm{PHB}} = 4.76\mathrm{l/g}$, $k_{\mathrm{RES}} = 2.81\mathrm{l/g}$) were taken from Franz (2015). Normally distributed random variables with zero mean and a variance of 0.1 are added to the generated data to simulate measurement noise. If not stated differently the initial values of the states are set as

$$\mathbf{x}_0 = [20 \ 1.5 \ 0.18 \ 0.125]^\mathsf{T}. \tag{35}$$

The following tuning parameters are maintained constant in each scenario. For the UKF, $\alpha = 0.001$, $\kappa = 0$, $\beta = 2$, $Q = \mathrm{diag}(10^{-12}, 10^{-12}, 10^{-12}, 10^{-12})$, and $R = 0.1$. For the MHE, $N = 10$, $\mathcal{R} = 1$ and $\mathcal{Q} = \mathrm{diag}(10^2, 10^4, 10^3, 10^3)$ where $\mathrm{diag}(a)$ is a diagonal matrix with the vector a in the diagonal. The sampling interval is 0.1 hours for both observers.

4.1 Optimal Scenario

Here, the performance of both state estimators is compared for an *optimal* scenario in which the estimators use the same initial states and parameters as the simulation. Thus, it is assumed that the initial conditions are known and only measurement noise is present. In this case, there is no initial estimation error, hence $P_0 = \mathrm{diag}(10^{-3}, 10^{-3}, 10^{-3}, 10^{-3})$. For the MHE the arrival cost matrix is $\mathcal{P} = P_0^{-1}$ which, for simplicity, is kept constant at all times. The results of these state estimations are shown in Figure 2. For both observers the estimated states $\hat{x}_k$ were in good agreement with the true state despite the measurement noise. Therefore, in the *optimal* scenario, both observers are functional.

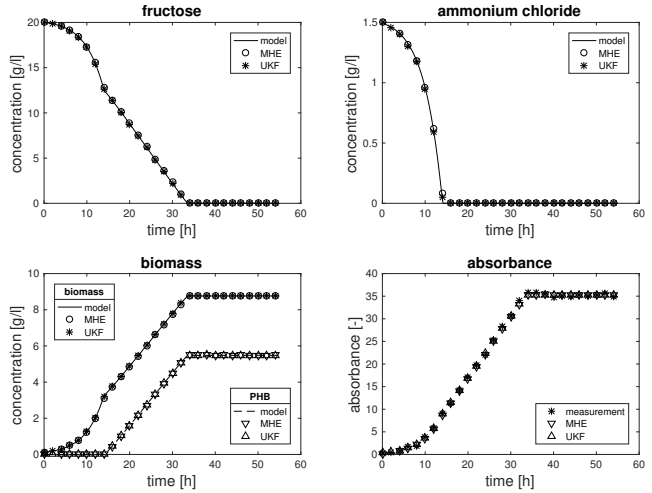

Fig. 2. Estimates for the *optimal* scenario.The dynamics of the states $\hat{x}_k$, estimated by both observers, the simulated state $\hat{x}(t)$ and the measurement of the absorbance A_k, which served as an input for the observers, as well as the estimated absorbance values $\hat{A}_k$ are shown.

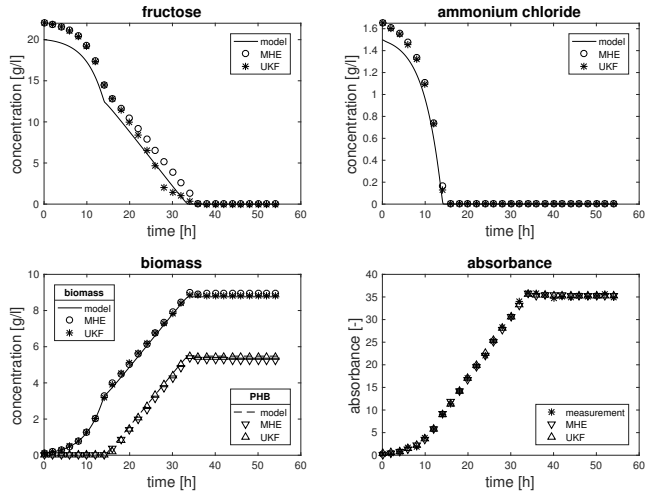

Fig. 3. Estimates for poorly guessed initial conditions.

4.2 Scenario with Unknown Initial Conditions

In an experimental context, the initial concentration are usually not known exactly, e.g. error in media preparation or inoculation. To test the performance of both observers in such a scenario, the observers were tested with an error of 10% on the actual initial substrate concentrations used by the model. In this case $P_0 = \mathrm{diag}(4, 0.0225, 0.001, 0.001)$ and $\mathcal{P} = \mathrm{diag}(1, 10^5, 10^5, 10^5)$ apply. Figure 3 shows that both estimators successfully converge to the actual state.

4.3 Impact of Measurement Equation

In this section, the impact of the measurement equation on the functionality of the observers is studied. In a previous work the measurement equations was calibrated offline for every experimental data set. As can be seen in Franz (2015), k_{PHB} and k_{res} differ for each data set and are therefore uncertain. Here, we analyze the influence of the

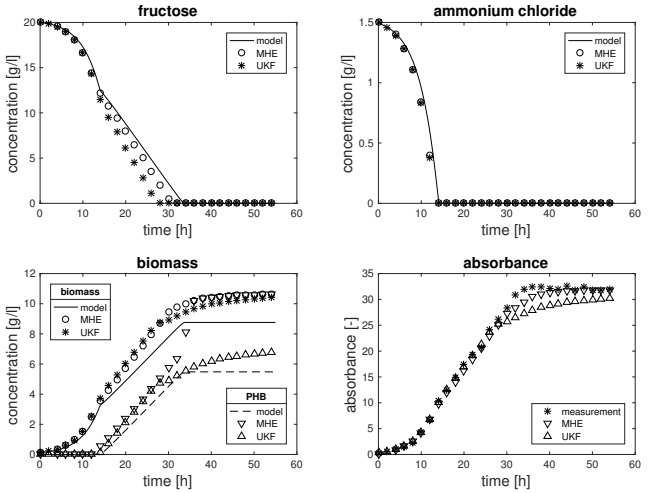

Fig. 4. Estimates with uncertain measurement equation parameters.

uncertain parameters on the functionality of the observers using two different sets of measurement parameters, for state estimation ($k_{\mathrm{PHB}} = 4.76\,\mathrm{l/g}$ $k_{\mathrm{RES}} = 2.8\,\mathrm{l/g}$) and simulation of the plant, respectively ($k_{\mathrm{PHB}} = 3.79\,\mathrm{l/g}$ and $k_{\mathrm{RES}} = 3.4\,\mathrm{l/g}$). As Figure 4 shows, the uncertainty in the measurement equation impairs the functionality of both observers in different extent. To further improve the performance of the observers, they were extended in a way, that allows to estimate the parameters (k_{PHB} and k_{res}) and the states simultaneously. For the observers, the initial parameter guesses are values randomly chosen within the parameter range shown in Franz (2015) and the initial concentrations are the same as in Equation (35).
The results of the simultaneous state and parameter estimation are shown in Figure 5. The overall performance of both observers significantly improved. The estimated parameters $\hat{k}_{\mathrm{PHB}}$ and $\hat{k}_{\mathrm{RES}}$ are not constant and differ from the calibrated parameter values determined by Franz (2015). The performance of the MHE observers was shown to be more robust for the whole investigated range.

5. CONCLUSION

In this work the performance of an UKF and a MHE was tested considering an HCM, which describes the PHB production in the organism *Cupriavidus necator*. A measurement equation was used to reconstruct the PHB concentration from the absorbance measurement of the culture broth.

Firstly, the observers were tested against uncertain initial conditions and additive measurement noise. The results showed that both observers converge to the modeled states satisfactorily. Then, we demonstrated that the uncertainty of the parameters of the measurement equation impairs the functionality of both observers. To overcome these uncertainties the observers were extended so as to achieve a simultaneous parameter and state estimation, which resulted in an improved performance of both observers. Considering these results, in principle both state observers can be used with HCMs for real-time process monitoring. However, choosing the appropriate state observer remains a trade-off between simplicity, robustness and the practicability regarding the real plant. The smaller computational

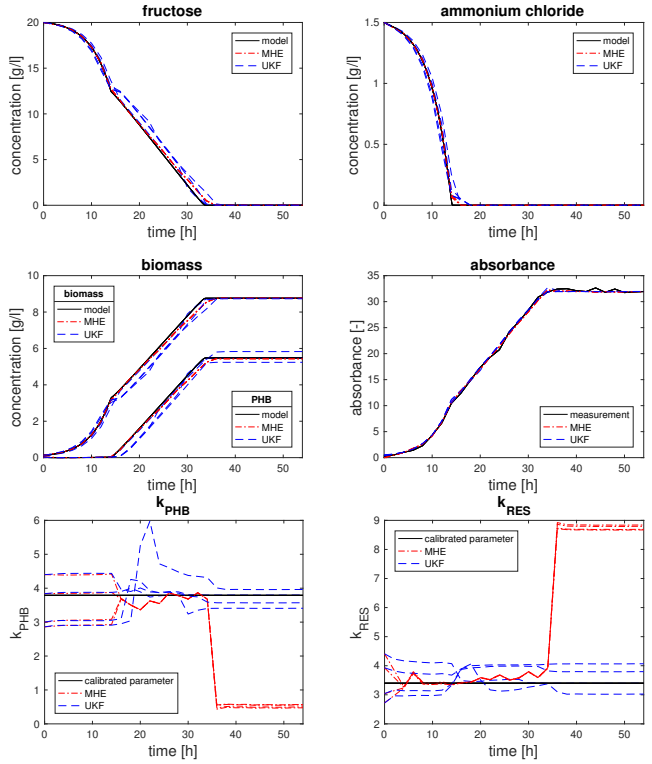

Fig. 5. Simultaneous estimation of states and uncertain parameters.

effort and the easier tuning of the UKF tend to allow a simple implementation which is of high importance for the industrial applications. However, due to the slow dynamics of biological processes, computational time does not play an important role. The MHE solves a optimization problem which requires a larger computational time but, as it was shown, the convergence is generally superior. Conversely, tuning the weighting matrices for the MHE can be critical. In general, the weak observability of the states hampers the robustness of both observers. Additional information, for instance, coming from delayed, time-sparse offline or indirect measurements needs to be taken into account. The MHE shows more flexibility in terms of using additional information, such as delayed measurements which can be handled easily and effectively with the MHE. This last point will be addressed in future research.

In general it can be concluded, that HCMs can be used for state observation and have a high potential to improve the performance of industrial biotechnological processes by facilitating process control and optimization.

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