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State Estimation of Microalgae Photobioreactors: Applications to *Haematococcus Pluvialis*

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Haematococcus Pluvialis is a type of freshwater green unicellular microalgae that can be used as economical natural source of astaxanthin. To guarantee optimal algae production and the proper conditions for the obtainment of high yield of astaxanthin, the growth process should be carefully monitored and controlled. When on-line measurements are not available, state estimators can provide a useful tool for process monitoring. In this paper a geometric observer is designed based on an extended version of the Droop model for microalgae growth, identified on the basis of batch experiments. The observer functioning and convergence are tested using experimental data obtained from a laboratory scale bioreactor.

1. Introduction

Microalgae production has attained increased attention during the last years. This is due on the one hand due their efficiency in producing e.g. lipids and carbohydrates (Visca et al., 2017), biodiesel and bioethanol (Peralta-Ruiz et al., 2018) or hydrogen (Nurdiawati et al., 2018), and on the other hand for the potential in wastewater treatment (Mezzanotte et al., 2018). In the present work the focus is put on the species Haematococcus pluvialis, a freshwater, green, unicellular microalgae that has received considerable scientific and biotechnological attention in recent years as a natural source of carotenoid astaxanthin, which possesses strong antioxidant capacities (Borowitzka, 2010). These algae pass through different cell stages and adapt their behaviour during growth, in particular under stress conditions. To guarantee the achievement of good performance and efficiency, the process requires advanced monitoring and regulation procedures, but the lack of accurate and reliable sensors can be an obstacle (Posten, Feng Chen, 2016, Havlik, et al., 2013, Bernard, 2011). As it is well-known from a system-theoretic point of view, software sensors or observers can provide efficient solutions to overcome this lack of information by applying adequate model-based data analysis schemes.
In the literature several approaches to efficiently estimate state variables in microalgae growth processes have been discussed. Among them are the high gain observer (Bernard, et al.,1999), a robust Luenberger observer (Benavides, et al., 2015), interval and Lipschitz observers (Goffaux et al., 2009, Khaksar Toroghi et al.,2013), and continuous-discrete Kalman Filters (Rocha et al., 2012, Jerono et al., 2018). In Jerono et al. (2018) additionally an analysis of the local observability properties of the Droop model with biased *on-line* measurement of the optical density (OD) and measurement of the extracellular nitrate concentration has been performed showing that the model is locally observable as long as the nitrate is not completely depleted.
These considerations motivate the question if a simple observer scheme can be designed which enables the fast (and robust) estimation of crucial state information for monitoring purposes. A possible approach to this question is provided by the geometric observer design (Alvarez, Lopez,1999, Tronci et al., 2005) which is based on the definition of an estimation structure consisting on a systematic choice of variables which are reconstructed from the measurement data and others which are estimated using the model only.
In the present work the growth of *Haematococcus Pluvialis* is investigated using an extended version of the Droop model for microalgae growth identified on the basis of batch experiments carried out in a 2 liter photo-

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bioreactor equipped with instruments for measuring OD, light intensity, dissolved oxygen partial pressure in the liquid phase and the exit gas, CO_2 in the exit gas, pH, and temperature. The model describes the dynamics of biomass, nitrate, cell intern content of nitrate, carbon dioxide and oxygen concentration. It is shown that the OD measurement is sufficient to obtain a convergent state estimate and a geometric observer is designed for a model-based state estimation. The performance of the observer is assessed using data from a batch experiment with additional off-line substrate measurements taken for validation purposes.

2. Problem statement

The Droop model and its extensions (Droop,1968, Bernard,2011, Mairet, Bernard,2016) have proved to provide an efficient means to capture the complex nonlinear interplay between the relevant process and biological variables. For this reason, in the present study the following extension from (Bernard, 2011) is considered in which additionally the gas balance is included

$$\dot{b} = -db + \mu(I_0, b, q, c)b, \qquad b(0) = b_0 \tag{1a}$$

$$\dot{q} = \rho(q, s) - \mu(I_0, b, q, c)q, \qquad q(0) = q_0 \tag{1b}$$

$$\dot{s} = d\,(s_{in} - s) - \rho(q, s)b, \qquad s(0) = s_0 \tag{1c}$$

$$\dot{c} = d\,(c^* - c) - k_l^c a\,(c - c^*) - (\gamma\,\mu(I_0, b, q, c) - r_o)b, \qquad c(0) = c_0 \tag{1d}$$

$$\dot{o} = d\,(o^* - o) - k_l^o a\,(o - o^*) + (\gamma\,\mu(I_0, b, q, c) - r_o)b, \qquad o(0) = o_0 \tag{1e}$$

In (1) the variables are time t, dilution rate d, biomass b, growth rate μ, nitrate concentration s with feed concentration s_{in}, internal quota q (i.e., nitrate content per biomass unit), substrate adsorption rate ρ, CO_2 concentration c and O_2 concentration o with saturation concentrations c^*, o^*, respectively.
The continuously measured output is assumed to be given by the turbidity (depending monotonically on the biomass) as well as the partial pressures pO_2 of dissolved oxygen in the liquid phase and in the exit gas and the partial pressure of CO_2 in the exit gas. Additional to these continuous on-line measurements sampled (off-line) substrate measurements are available, which can be used for quality monitoring, model adaptation and validation purposes. In the model (1) the O_2 production is assumed proportional to the biomass growth rate and equal to the CO_2 consumption rate. Over the biomass interval of interest, the turbidity can be approximated as a linear function of the biomass. Assuming that the exit gas partial pressure of CO_2 is identical to the ones in the reactor gas volume Henry's law can be used to uniquely determine the concentrations of carbon-dioxide in the reactor. Equivalently, the concentration of dissolved oxygen can be uniquely obtained from the pO_2 measurement. This implies that the output map $\boldsymbol{y}$ can be uniquely inverted to establish a (pseudo) measurement

$$\boldsymbol{y} = [b, c, o\,]^{\boldsymbol{T}}.$$

The preceding system is written in compact form as

$$\dot{\boldsymbol{x}} = \boldsymbol{f}(\boldsymbol{x}, \boldsymbol{d}),\ \boldsymbol{x}(\boldsymbol{0}) = \boldsymbol{x_0},\quad \boldsymbol{f} = [\,f_1, f_2, f_3, f_4, f_5]^{\boldsymbol{T}},\ \boldsymbol{x} = [b, q, s, c, o\,]^{\boldsymbol{T}} \tag{2a}$$

$$\boldsymbol{y} = C\boldsymbol{x}, \qquad C = \begin{bmatrix} 1 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 1 & 0 \\ 0 & 0 & 0 & 0 & 1 \end{bmatrix} \tag{2b}$$

One of the main factors in microalgae growth on the basis of photosynthesis is the light intensity I in the reactor as well as the CO_2 content. In virtue of the Beer-Lambert law the light intensity in the reactor depends on the turbidity so that $\boldsymbol{I} = \boldsymbol{I_0 e^{-\alpha\, b}}$. Considering a Monod-type dependency of μ on the concentration c and the standard Droop growth rate function in dependence of the internal quota q it follows that

$$\mu(I_0, b, q, c) = \bar{\mu}\frac{c}{c + k_c}\left(1 - \frac{q_{min}}{q}\right) I_0 e^{-\alpha\, b}.$$

The substrate absorption rate ρ on the other hand is modeled by a Monod-type dependency with inhibition

$$\rho(q, s) = \rho_{max}\frac{s}{s + k_s}\left(1 - \frac{q}{q_{max}}\right).$$

The considered oxygen production rate in (1) is an adaption from the one employed in (Li, et al., 2003).
The problem considered in this paper consists in designing an algorithm which provides a state estimate $\hat{\boldsymbol{x}}$ that asymptotically converges to the real state $\boldsymbol{x}$, sufficiently fast for monitoring and possible feedback control purposes. In particular, as can be seen from (1) for the regulation of e.g. the biomass production rate the

substrate concentration is not necessary and only the internal substrate quota q must be known. Thus, it is sufficient to estimate the substrate for monitoring purposes only.

3. Model validation

The experimental set-up for the batch process in the 2 *l* photo-bioreactor Labfors from InforsHT is described in (Jerono et al., 2018). During the batch experiment the CO_2 concentration in the exit gas remained practically constant at 2% corresponding to $\boldsymbol{c}^*$. Accordingly, it is hard to identify kinetic parameters of the CO_2 dependency from the given data as it remains almost constant. Thus it has been included in the maximum growth rate according to

$$\bar{\mu}^* = \bar{\mu}\frac{c^*}{c^*+k_c}$$

which has been identified from the experimental data of the biomass growth. In Figure 1 a comparison of the four states $[b, q, s, o]^T$ with discrete-time measurements of the biomass (through OD), nitrate concentration and pO_2 in the liquid phase are shown. The associated parameter values are

$$\bar{\mu}^* = 6.7\text{E}{-}03\ \frac{m^2 s}{\mu mol\, h},\ I_0 = 45\ \frac{\mu mol}{m^2\, s},\ \alpha = 3.3802\ \frac{l}{m^2},\ q_{min} = 0.8436\ \frac{\text{mgN}}{mgC},\ q_{max} = 1.3909\ \frac{\text{mgN}}{mgC},$$

$$\rho_{max} = 0.0236\frac{1}{h},\ s_{in} = 0.567\ \frac{\text{mg}}{l},\ k_s = 0.009\ \frac{\text{mg}}{l},\ k_l^o a = 51.0908\ \frac{1}{h},\ k_l^c a = 0.91\, k_l^o a,$$

$$o^* = 6.63\ \frac{mg}{l},\quad c^* = 0.51\ \frac{\text{mg}}{l},\ \gamma = 3.4579E03\ \frac{\text{mgO}_2}{mgC},\ r_o = 0.1002\frac{1}{h}$$

Note that in the parameter identification the substrate has been normalized to the concentration in pure medium (i.e., 1000 mg/l). It can be seen that for the biomass and substrate the model fits well with the data, while for the oxygen concentration only the qualitative behavior is recovered. This is probably due to unmodeled (i.e., parasitic) dynamics having an influence on the pO_2 measurement. Possible sources for such behavior are e.g. biofouling on the optical sensor device or pressure changes in the reactor due to clogging of the exit gas filter.

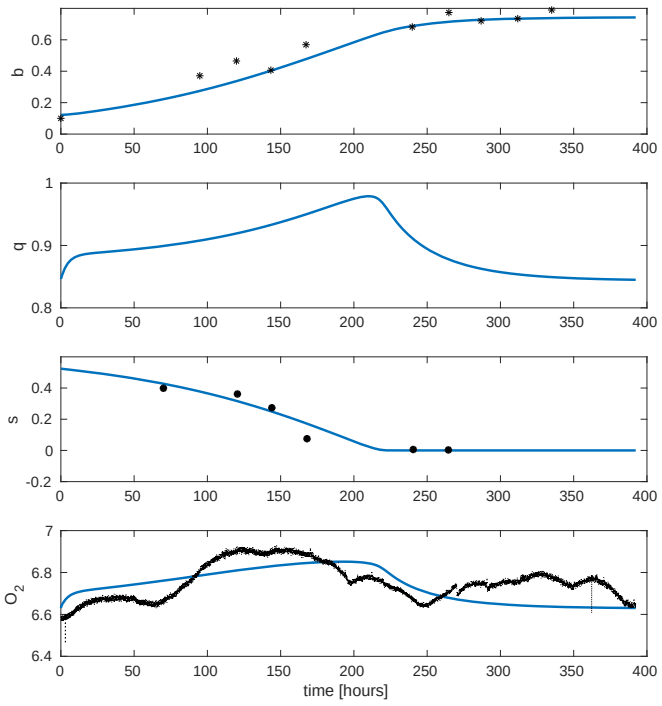

Figure 1: Simulation (blue) and measurements (black) of the batch data with biomass (b), quota (q), substrate (s) and dissolved oxygen (O_2) concentrations.

4. Observer design

For the purpose of state estimation first the local observability properties must be established in order to define an adequate estimation structure.

4.1 Observability properties

From the previous studies (Moreno et al., 2014, Schaum, Meurer, 2015, Jerono, et al., 2018) on the observability properties of the Droop model (and extensions of it), it is known that the Droop model without the inhibition terms and gas balance is globally observable (Moreno et al.,2014, Schaum, Meurer, 2015). If the inhibition is present, the system is detectable as long as the dilution rate does not vanish (i.e., $d > 0$). As for $d = 0$ the nitrate concentration s converges to 0, the states can always be asymptotically reconstructed. Furthermore, the model is locally observable as long as the nitrate is not completely depleted (Jerono, et al., 2018). Nevertheless, for small nitrate concentrations s the observability map becomes ill-conditioned and thus a reconstruction of s from the measurement data lacks robustness against modeling errors and measurement noise. On the other hand, given the monotonic dependency of the function $\boldsymbol{\mu}(\boldsymbol{I_0}, \boldsymbol{b}, \boldsymbol{q}, \boldsymbol{c})$ on the quota q, this can be robustly estimated using either a biomass or dissolved oxygen measurement. The substrate concentration on the other hand can be estimated asymptotically. In the sequel the biomass concentration is used for state estimation given that (i) the oxygen measurement presents too many parasitic effects and (ii) irregularities due to the onset of biofilm production can be better monitored using the additional off-line OD measurements.

4.2 Geometric observer

Consider the partition of the state $\boldsymbol{x}$ according to

$\boldsymbol{x} = [\boldsymbol{x_o}, s]^{\boldsymbol{T}},\ \boldsymbol{x_o} = [b, q, c, o]^{\boldsymbol{T}}.$

Introduce the transformed state vector

$\boldsymbol{z} = [\boldsymbol{z_o}, s]^{\boldsymbol{T}} = \boldsymbol{\Phi}(\boldsymbol{x}),\ \boldsymbol{z_o} = \boldsymbol{\mathcal{O}_b}(\boldsymbol{x}),\ \boldsymbol{\mathcal{O}_b}(\boldsymbol{x}) = [b, f_1, c, o]^{\boldsymbol{T}}$

where $\boldsymbol{f_1}$ is defined in (2a). The dynamics for the observable components is given by

$\dot{\boldsymbol{z_o}} = \boldsymbol{\varphi_o}(\boldsymbol{z_o}, s, d), \qquad \boldsymbol{z_o}(\boldsymbol{0}) = \boldsymbol{z_{o,0}}$

$\boldsymbol{y} = \boldsymbol{C_o}\,\boldsymbol{z_o}\ .$

Set the geometric observer

$$\dot{\hat{\boldsymbol{z}}}_{\boldsymbol{o}} = \boldsymbol{\varphi_o}(\hat{\boldsymbol{z}}_{\boldsymbol{o}}, \hat{s}, d) - \boldsymbol{L}(\boldsymbol{C_o}\hat{\boldsymbol{z}}_{\boldsymbol{o}} - \boldsymbol{y}), \qquad \hat{\boldsymbol{z}}_{\boldsymbol{o}}(\boldsymbol{0}) = \hat{\boldsymbol{z}}_{\boldsymbol{o,0}} \tag{3a}$$

$$\dot{\hat{s}} = d\,(s_{in} - \hat{s}) - \rho(\hat{q}, \hat{s})y_1, \qquad \hat{s}(0) = \hat{s}_0 \tag{3b}$$

with the correction gain matrix L given by

$$\boldsymbol{L} = \begin{pmatrix} l_1 & 0 & 0 \\ l_2 & 0 & 0 \\ 0 & l_3 & 0 \\ 0 & 0 & l_4 \end{pmatrix}$$

and chosen in order to prevent unnecessary cross-correlations in the estimation error dynamics. The observer convergence can be ensured by choosing l_3, l_4 sufficiently large and l_1, l_2 so that the eigenvalues of the matrix

$$\boldsymbol{M} = \begin{pmatrix} -l_1 & 1 \\ -l_2 & 0 \end{pmatrix}$$

are located sufficiently far in the left half complex plane and at the same time noise amplification is reduced. The actual state estimate $\hat{\boldsymbol{x}}$ is obtained through the inverse transformation

$$\hat{\boldsymbol{x}} = \boldsymbol{\Phi^{-1}}(\hat{\boldsymbol{z}}), \quad \hat{\boldsymbol{z}} = [\hat{\boldsymbol{z}}_{\boldsymbol{o}}^{\boldsymbol{T}}, s]^{\boldsymbol{T}}. \tag{4}$$

Note that equivalently the inverse (i.e., original) dynamics

$$\dot{\hat{\boldsymbol{x}}}_{\boldsymbol{o}} = \boldsymbol{f_o}(\hat{\boldsymbol{x}}_{\boldsymbol{o}}, \hat{s}, d) - \boldsymbol{\Omega_b^{-1}}(\hat{\boldsymbol{x}}_{\boldsymbol{o}}, \hat{s})\boldsymbol{L}(\boldsymbol{C_o}\hat{\boldsymbol{x}}_{\boldsymbol{o}} - \boldsymbol{y}), \qquad \hat{\boldsymbol{z}}_{\boldsymbol{o}}(\boldsymbol{0}) = \hat{\boldsymbol{z}}_{\boldsymbol{o,0}}, \qquad \boldsymbol{\Omega_b^{-1}}(\hat{\boldsymbol{x}}_{\boldsymbol{o}}, \hat{s}) = \frac{\partial \mathcal{O}_b}{\partial x_o}$$

$$\dot{\hat{s}} = d\,(s_{in} - \hat{s}) - \rho(\hat{q}, \hat{s})y_1, \qquad \hat{s}(0) = \hat{s}_0$$

can be employed for estimation directly.

4.3 Observer evaluation

The observer has been implemented for the model reduced by the CO_2 component, given that the associated measurement in the exit gas remained constant over the process. Given that the on-line OD measurement is biased due to biofouling on the sensor device (Jerono et al., 2018) for the observer evaluation the following measurement data is used: (i) the validated simulated biomass as virtual measurement artificially subjected to noise, and (ii) the on-line measurement of the dissolved oxygen. The observer gains are set to $l_1=l_2=0.0005$, $l_4=0.5$. The result is shown in Figure 2 where it can be seen that the states converge quickly in the estimated biomass and quota components, with the biomass presenting basically a low-pass filtered version of the measurement and the quota estimate presenting some noise amplification. The substrate is estimated on a detectability time-scale for monitoring purposes only. The oxygen concentration is reasonably recovered presenting a compromise between the model prediction and the actual measurement (subject to unmodeled dynamics).

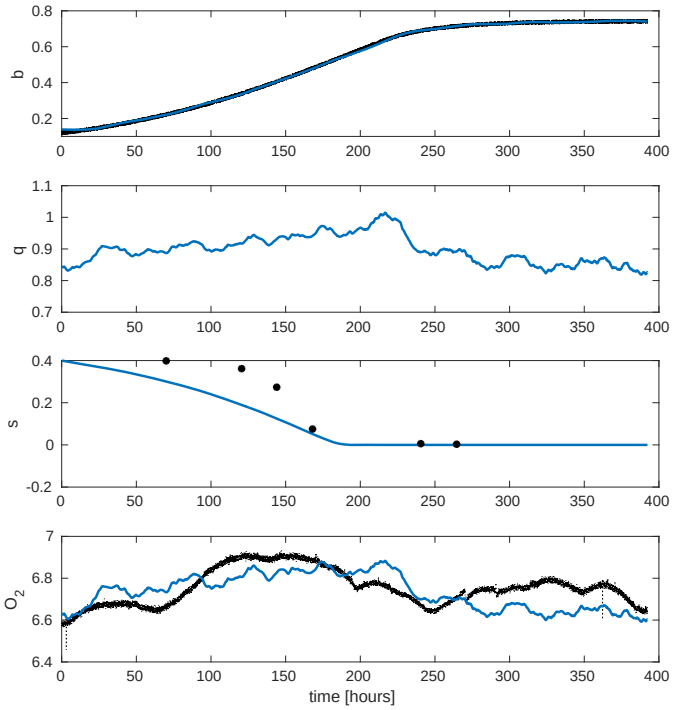

Figure 2: State estimates (blue) and measurement data (black) for the batch process with biomass (b), quota (q) substrate (s) and oxygen (O_2) components.

5. Conclusions

A validated model for the growth of the microalgae *Haematococcus pluvialis* is presented and used for the design of a geometric observer for state estimation. The observer is evaluated using batch data showing a good performance for the estimation of biomass, internal substrate quota, external substrate concentration and dissolved oxygen concentration in the reactor.
The presented results show the possibility to employ the presented estimator for feedback control purposes in a chemostat (or turbidostat) operation. The continuous reactor operation will be evaluated in future studies.

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