ADAPTIVE BIOMASS OBSERVER ON THE BASIS OF MEASUREMENTS OF OXYGEN

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ABSTRACT

A new adaptive asymptotic biomass observer for fed-batch *E. coli* growth on glucose is proposed. The observer uses on-line measurements of oxygen and stirrer speed only. The observation algorithm includes a procedure of on-line estimation of yield coefficients on the basis of off-line measurements of biomass concentration. Simulation investigations of the observer are carried out using experimental data as input information. The observation algorithm is verified through laboratory experiment of a recombinant *E. coli* strain.

I. INTRODUCTION

Escherichia coli is a frequently used host organism for production of recombinant proteins. It has many advantages, such as being well-characterized and supporting growth to high cell densities, but also has some drawbacks. One of the difficulties encountered in *E. coli* cultivation is the formation of the metabolic by-product, acetate, in case of excess glucose under aerobic conditions. Accumulation of oxidative acetate reduces both cell growth and recombinant protein production. The accumulation of acetate and its inhibiting effects is reduced by applying an optimal glucose feeding profile during the fed-batch part of cultivation [2]. The feeding rate, F_{in}, is calculated on-line for each time interval using the following expression:

$$F_{in}(t) = \frac{\mu_{set} V}{Y_{xg} S_{in}} \left[X(t_0) e^{\mu_{set}(t-t_0)} \right] \qquad (1)$$

where Y_{xg} is biomass/glucose yield coefficient; t_0 is the start of the interval; S_{in} is glucose concentration in feed; V is the volume and μ_{set} is a set value of specific growth rate. As obvious from above, the fed rate depends mostly on biomass concentration in the reactor. Unfortunately, the real growth information is not used due to the lack of cheap and reliable on-line biomass sensors. Instead, a predicted value of biomass concentration is applied that is calculated by the shown expression in the square brackets in (1).

The non-linear system control theory proposed an option for indirect biomass measurements design. The biomass observation can be obtained by combining the information from existing sensors using parameter and state estimation [3-7]. As the process behavior is non-linear and time varying, usually an adaptive algorithm for biomass observation is proposed.

To address this matter, the paper presents a design of adaptive biomass observer during the fed-batch fermentation where the optimal profile of glucose feeding (1) is applied. That feeding strategy stabilizes the specific glucose rate at a critical value, q_S^{crit}. Hence, the concentrations of glucose and those of acetate are considered to be zero in the reactor. At the same time, the concentration of the other main substrate, oxygen, is kept at a constant value. Hence, the biomass grows on substrate feedings only because both limiting substrates are kept at constant concentrations in the reactor.

II. MODELS OF AEROBİC GROWTH OF *E. COLİ*

II.1. Models Of Aerobic Growth

For the considered process, M. Akensson proposed a biochemical model [1,2]. As the concentration of acetate is kept zero in the reactor, the model can be reduced and the dynamics of the main process variables during the fed-batch part of cultivation can be presented as follows:

$$\begin{aligned}
&\frac{dV}{dt} = F_{in};\\
&\frac{d(S.V)}{dt} = -q_S(X.V) + F_{in} S_f;\\
&\frac{d(X.V)}{dt} = \mu(X.V);\\
&\frac{d(C_O V)}{dt} = -q_O(X.V) + K_{La}(N).V.(C_O^* - C_O).
\end{aligned} \qquad (2)$$

where $q_S = q_S^{\max} \dfrac{S}{k_S + S}$ is specific glucose uptake rate.

The feeding strategy keeps the value of $\boldsymbol{q_S}$ above or equal to the critical one, $\boldsymbol{q_S \geq q_S^{crit}}$. The values of specific growth rate therein, $\boldsymbol{\mu}$, and of specific oxygen uptake rate, $\boldsymbol{q_o}$, are calculated using the expressions:

$$\begin{aligned} \mu &= q_S^{crit} Y_{SX}^{oxid} + (q_S - q_S^{crit}) Y_{SX}^{ferm} \\ q_O &= q_S^{crit} Y_{OG} \end{aligned} \tag{3}$$

In the model (2), the oxygen dynamics is presented by the dissolved oxygen concentration through Henry's law

$$O = H.C_O \tag{4}$$

and the volumetric oxygen transfer coefficient, K_{La}, is presented as a function of the stirred speed:

$$K_{La} = \alpha.(N - N_o) \text{ where } N > N_o \tag{5}$$

Given the existing sensors, the available process information consists of on-line measurements of oxygen, O_2, and stirrer speed, N; glucose feed rate, F_{in}, and glucose concentration in feed, S_f; This available on-line information is used for biomass observer design.

II.2. General Dynamical Model

G. Bastin and D. Dochain proposed a method for deriving a General Dynamical Model for bioreactor [3]. The model is an operational one and translates the available process information into appropriate inputs for biomass observer design. The model is derived from the simplest description of a biotechnological process - process reaction scheme. Once the reaction scheme is available, the model derivation can be made fully systematic by applying the rules proposed in [3].

An appropriate reaction scheme is proposed following the process dynamics (2). For the case under consideration, the optimal profile (1) guarantees acetate production restriction as well as glucose uptake rate saturation. The glucose and acetate concentrations are close to zero in the reactor. The oxygen concentration is kept at a constant value (30%) and all transferred oxygen is used for degradation of fed glucose. Hence, the reaction scheme consists of two reactions The first one, φ_1, is constant with specific uptake rate q_S^{crit} and the second reaction, φ_2, represents the rate $(q_S - q_S^{crot})$ in (3). In the case under consideration, $q_s \geq q_s^{crit}$, both reactions are activated. The process scheme is as follows

$$\begin{aligned} S + O_2 &\xrightarrow{\varphi_1} X \\ S + O_2 &\xrightarrow{\varphi_2} X \end{aligned} \tag{6}$$

The General Dynamical Model of the process is derived according to the scheme (5).

$$\begin{bmatrix} \dot{S.V} \\ \dot{X.V} \\ \dot{C_O V} \\ \dot{OUR.V} \end{bmatrix} = \begin{bmatrix} -1 & -1 \\ k_1 & k_2 \\ k_3 & 0 \\ k_4 & k_5 \end{bmatrix} \begin{bmatrix} \varphi_1 \\ \varphi_2 \end{bmatrix} + \begin{bmatrix} F_{in} S_f \\ 0 \\ Q_{in} V \\ 0 \end{bmatrix} \tag{6}$$

The model (6) includes the dynamics of oxygen uptake rate, $OUR.V$, as a measured process variable. The measurements of oxygen uptake rate can be calculated by the expression

$$OUR^{meas} V = Q_{in}^{meas} V - F_{in}.C_O^{meas}. \tag{7}$$

when the oxygen concentration is constant.

The operational model (6) is used for biomass observer design.

II.3. Biomass observer design

The general model (6) consists of two main parts. The first term represents process the kinetics and the second one represents the transport dynamics. In the case under consideration, the process kinetics is unknown, and the transport dynamics is known and must be used. For this purpose a transformation of model (6) has to be made in such a way that the dynamics of the process to be presented with known information only, namely measured variables and transport dynamics. Hence, the available process information consists of: measurements of the oxygen concentration, $C_O V$, and oxygen uptake rate, $OUR.V$; and the known information of the transport dynamics (terms $Q_{in}V$ and $F_{in}S_f$)

The model transformation is made applying the basic property of General Dynamical Model [3]. According to that property, there exists a state transformation

$$Z = A_0 \xi_a + \xi_b \tag{8}$$

where A_0, is the unique solution to the matrix equation,

$$A_0 K_a + K_b = 0 \tag{9}$$

such that the state-space model (6) is equivalent to

$$\begin{aligned} \dot{\xi}_a &= K_a \varphi(\xi_a, \xi_b) - D\xi_a + F_a \\ \dot{Z} &= A_0 F_a + F_b \end{aligned} \tag{10}$$

State variables of model (6) are divided into measured variables, $\xi_{a,}$, and unmeasured ones, ξ_b, as follows:

$$\xi_a = \begin{bmatrix} C_0.V \\ OUR.V \end{bmatrix}; \xi_b = \begin{bmatrix} S.V \\ X.V \end{bmatrix}, \tag{11}$$

and the appropriated matrises are defined:

$$K_a = \begin{bmatrix} -k_3 & 0 \\ k_4 & k_5 \end{bmatrix} \text{and } K_b = \begin{bmatrix} -1 & -1 \\ k_1 & k_2 \end{bmatrix};$$

$$F_a = \begin{bmatrix} Q_{in}.V \\ 0 \end{bmatrix} \text{ and } F_b = \begin{bmatrix} F_{in}S_f \\ 0 \end{bmatrix}.$$

According to (9), the matrix A_o is obtained

$$A_0 = \frac{-K_b}{K_a} = \begin{bmatrix} \frac{k_1 - k_2}{k_2 k_3} & \frac{1}{k_2 k_3 q_s^{crit}} \\ 0 & -\frac{1}{k_3 q_s^{crit}} \end{bmatrix} \tag{12}$$

and the expressions for the auxiliary variables, Z and its dynamics are calculated

$$\begin{aligned} Z_1 &= \frac{k_1 - k_2}{k_2 k_3} C_o V + \frac{1}{k_2 k_3 q_s^{crit}} OUR.V + S.V; \\ Z_2 &= -\frac{1}{k_3 q_S^{crit}} OUR.V + XV. \end{aligned} \tag{13}$$

$$\begin{aligned} \frac{dZ_1}{dt} &= \frac{k_1 - k_2}{k_2 k_3} Q_{in}.V + F_{in} S_f; \\ \frac{dZ_2}{dt} &= 0; \end{aligned} \tag{14}$$

Taking into account that $S=0$ and after some appropriate substitutions, the auxiliary variable $\boldsymbol{Z_2}$ is presented as a function of $\boldsymbol{Z_1}$

$$Z_2 = -k_2 \left(Z_1 - \frac{k_1 - k_2}{k_2 k_3} C_0 V \right) + X.V \tag{15}$$

Therefore, using the equations (6), (14) and (15) the biomass observer for the considered case is derived as follows:

$$\begin{aligned} \frac{dV}{dt} &= F_{in}; \\ \frac{dZ_1}{dt} &= \frac{k_1 - k_2}{k_2 k_3} Q_{in}.V + F_{in} S_f; \\ \frac{dZ_2}{dt} &= 0; \end{aligned} \tag{16a}$$

$$\hat{X} = \left(Z_2 + k_2 Z_2 - \frac{k_1 - k_2}{k_3} C_O V \right) / V. \tag{16b}$$

The observation algorithm consists of three main steps. In the first one, the values of auxiliary variables and liquid volume are calculated by differential equations (16a). A MATLAB S-Function is applied for this purpose. In the second step, a observation of biomass is obtained by the expression (16b). This expression is a function of the of yield coefficients, k_1 ,k_2, k_3, that are unknown and time-varying. Therefore, a parameter estimation algorithm is proposed as a third step. In this step, estimates of the yield coefficients are made by comparison of the observed and off-line measured values of biomass. After an optimization procedure, the appropriate values of the coefficients are obtained.

III. SİMULATİON İNVESTİGATİONS

The experimental data of four fermentations of *E.coli* are used for simulation investigations of the proposed observers. The results are shown on next figures where the observations of biomass are presented with lines, and the measured points with stars.

In Figures 1 and 2, the simulations with the fermentation data No 43 and 48 are shown respectively.

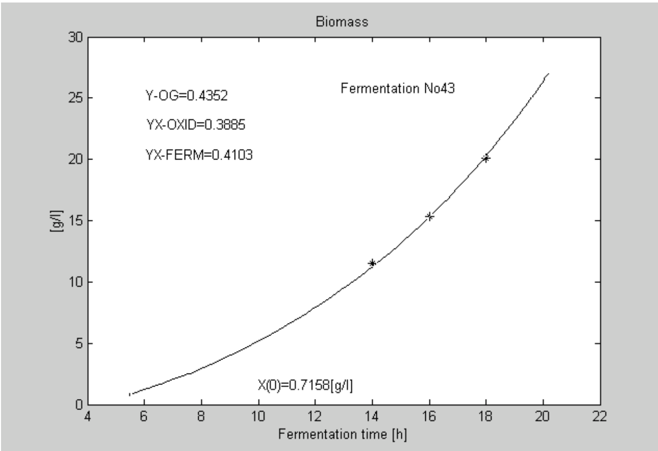

Figure 1 – Fermentation No 43

As can be seen in the figures, the observation curves are very close to the off-line measured points of biomass.

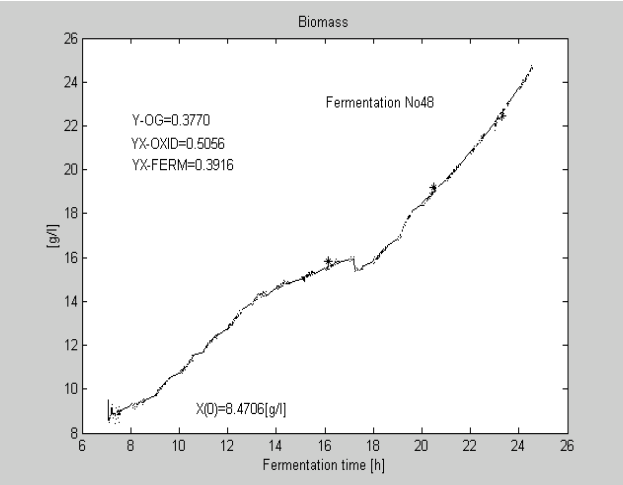

Figure 2 – Fermentation No

These results prove that the feeding profiles are the really optimal ones and they stabilize the process close to the critical value of the glucose uptake rate. Therefore, the acetate production as well as the glucose concentrations are close to zero in the reactor as is was assumed in the observation algorithm. Moreover, in Figure 2, a jump down of observation curve recognizes a change of the set value of the specific growth rate, $\mu_{set,}$ (from 0.125 to 0.1 h^{-1}) at 15.46 h of fermentation No 48. In this case again, the observation curve fits well with the measurements.

In Figures 3 and 4, the simulations with fermentations No 46 and 47 data are shown respectively. The observation curves are at a distance from the measured points. Perhaps, the off-line measurements are not made precisely, or the feeding profiles are not the optimal ones and some acetate is produced during the fermentations, however, the acetate and the glucose concentrations are not measured, therefore these are only hypothetical conclusions.

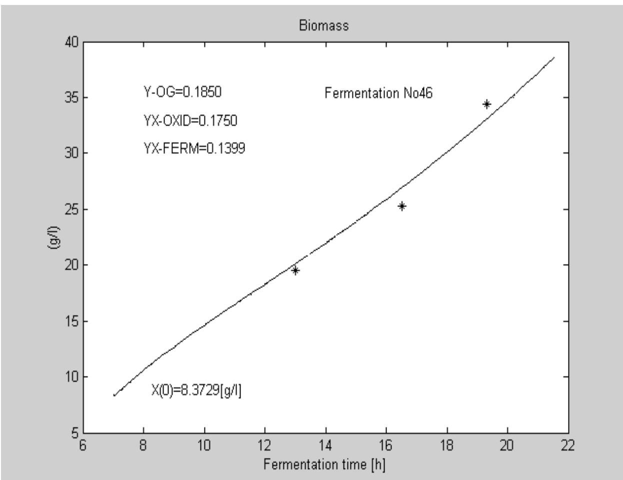

Figure 3 – Fermentation No 46

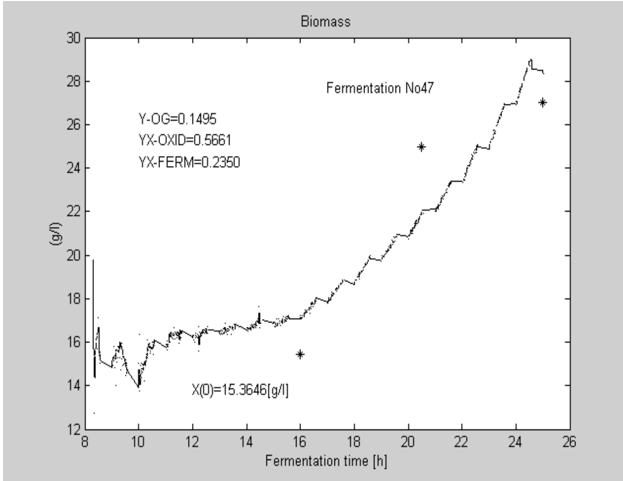

Figure 4 – Fermentation No 47

In general, the simulation investigations proved the lack of experimental reproducibility of the culture. Therefore, the feeding strategy proposed in [2] could not be considered as the optimal one for all experiments.

The results shown in all figures demonstrate the ability of the proposed adaptive algorithm to produce biomass observations on the basis of oxygen measurements. Better observations are obtained in the cases of optimal feeding strategies.

IV. EXPERİMENTAL İNVESTİGATİON

An experiment in continuous mode was carried out on the same strain in the laboratory. In this way, the experimental value of biomass/glucose yield coefficient, $\mathbf{Y}_{\mathbf{xs}}^{\mathbf{exper}}$, was obtained. It was equal to 0.41 h^{-1}. The estimated values of the same coefficient are obtained during the simulation investigations applying the expression $Y_{xs}^{estim} = (Y_{xs}^{oxid} + Y_{xs}^{ferm})/2$. It is observed that the coefficient keeps having a constant value, which, however, is different for each fermentation (see table).

A comparison between experimental, $\mathbf{Y}_{\mathbf{xs}}^{\mathbf{exper}}$=0.4100, and estimated, $\boldsymbol{Y}_{\boldsymbol{xs}}^{\boldsymbol{estim}}$, values shows that three out of the four estimated values are in proximity to the experimental one

Table

Fermentation	$\boldsymbol{Y}_{\boldsymbol{xs}}^{\boldsymbol{estim}}$ [h^{-1}]
No 43	0.3994
No 46	0.1575
No 47	0.4001
No 48	0.4130

That result shows the ability of the proposed algorithm to estimate also the unknown yield coefficients.

V. CONCLUSION

The proposed biomass observer is an adaptive asymptotic one. As the *E. coli* fermentation is a non-linear process with time varying parameters, a parameter estimation procedure is included in the observation algorithm. The values of process parameters are estimated at the moment when the biomass measurements are received as additional off-line information. Better results could be obtained if those three points are being measured at the beginning of the fed-batch part of the cultivation. Such off-line information would be good enough for biomass observer tuning The values of biomass/glucose yield coefficients calculated during the investigations are verified by continuous fermentation of the same strain. The different values of coefficients are obtained for each experiment. This fact proves the lack of experimental reproducibility of the culture, the later being the reason for applying an adaptive algorithm for biomass observation.

The observer of biomass could be considered as a key step to process control design. Thereupon, several interesting tacks can to be solved. Using the biomass observer, the next steps would be:
- the observed value of biomass as well as the estimated value of the yield coefficient $\boldsymbol{Y_{xs}^{estim}}$ could be used for calculation of the feeding profile of glucose (1) instead of the theoretical ones that are in the use in the laboratory;
- on the other hand, the observer of biomass could be considered as the first step of a closed loop adaptive linearizing control design of the glucose feeding and stirrer speed.

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