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ESTIMATION OF BIOMASS CONCENTRATION AND MULTIPLE SPECIFIC GROWTH RATES OF FED-BATCH FERMENTATION OF RECOMBINANT E. COLI

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Abstract: In this paper, a two step estimation approach is proposed for estimation of multiple (specific) growth rates and biomass concentration of fed-batch fermentation of recombinant E. coli. It is considered the case when the process is described using a switch between two partial sub-models. On the first step two out of the three growth rates are estimated on the basis of observer-based estimators using on-line measurements of acetate concentration. In the second step, the third biomass growth rate and the biomass concentration, are estimated for both submodels, on the basis of estimation algorithms using on-line measurements of glucose concentration. The performances of the proposed estimation algorithms are investigated by simulations for a model of fed-batch fermentation of recombinant E. coli.

Keywords: estimation, multiple growth rates, biomass concentration, fed-batch recombinant E. coli process

I. *Introduction*

Successful operation and control of recombinant fermentation processes requires accurate on-line knowledge about the real process state, including information about the main state variables and parameters. In many practical cases, it is impossible to measure the state variables and parameters directly becoming their estimation a necessary step. A possible approach to overcome this problem is to apply 'software sensors'[1-7].

This paper is dedicated to the estimation of the multiple biomass growth rates and the biomass concentration of a fed-batch fermentation of recombinant *E. coli*, which is characterized by the following reaction network [3]:

$$S + DO \xrightarrow{\mu_1} X + CO_2 \quad (1)$$

$$S \xrightarrow{\mu_2} X + CO_2 + A \quad (2)$$

$$A + DO \xrightarrow{\mu_3} X + C \quad (3)$$

where S is the glucose; DO – dissolved oxygen; X – biomass; A –acetate, which can be product or substrate depending on the metabolic pathway; CO_2 – carbon dioxide; μ_1, μ_2 and μ_3 are the specific growth rates for the three metabolic pathways. In the sequel DO, X, A, S, CO_2 will denote the concentrations of these variables. Pathways (1), (2), (3) refer to the respiratory growth on glucose (oxidative pathway), fermentative growth on glucose (reductive pathway) and the respiratory growth on acetate (oxidative pathway), respectively. In this paper (in contrast to [3]) maintenance reaction is omitted since the maintenance coefficient is considered as a constant.

II. *Problem Statement*

A general dynamical model of a stirred tank reactor [2] has the form:

$$\frac{d\boldsymbol{\xi}}{dt} = \mathbf{K}\,\mathbf{r}(\boldsymbol{\xi}) - D\boldsymbol{\xi} + \mathbf{F} - \mathbf{Q} \quad (4)$$

where $\boldsymbol{\xi}$ is the vector of state variables with dim(ξ)=n; $\mathbf{K}$ - the n×m yield coefficient matrix; D - the dilution rate; $\mathbf{F}$ - the feed rate vector with dim(F)=n; $\mathbf{Q}$ - the gaseous outflow rate vector with dim(Q)=n; $\mathbf{r}(\boldsymbol{\xi})$ - a reaction rate vector with dim(r)=m.

The dynamics of the fed-batch fermentation of recombinant *E. coli*, which is characterized by the reaction scheme (1) - (3), can be presented in the matrix form (5), by the following vectors and matrix:

$$\xi = \begin{bmatrix} X \\ S \\ A \\ O \\ C \end{bmatrix}; \mathbf{K} = \begin{bmatrix} 1 & 1 & 1 \\ -k_1 & -k_2 & 0 \\ 0 & k_3 & -k_4 \\ -k_5 & 0 & -k_6 \\ k_7 & k_8 & k_9 \end{bmatrix}; \frac{dW}{dt} = F_{in}; \; D = \frac{F_{in}}{W}$$

$$\mathbf{F} = \begin{vmatrix} 0 & DS_{in1} & 0 & OTR & 0 \end{vmatrix}^T; \mathbf{Q} = \begin{vmatrix} 0 & 0 & 0 & 0 & CTR \end{vmatrix}^T$$

$$r(\xi) = \begin{vmatrix} \mu_1 X & \mu_2 X & \mu_3 X \end{vmatrix}^T = \begin{vmatrix} \varphi_1 & \varphi_2 & \varphi_3 \end{vmatrix}^T \quad (5)$$

where $k_1 \div k_9$ are yield coefficients, OTR is the oxygen transfer rate, CTR is the carbon dioxide transfer rate, S_{in1} is the glucose concentration in the feed, W is the culture medium weight, F_{in} the influent flow rate For the system (4), (5), it is assumed that:

A1. The measurements of S, A, DO, CO_2, oxygen transfer rate, OTR, and the carbon dioxide transfer rate, CTR are measured on-line, while the biomass concentration is not measurable on-line.

A2. The elements of the yield coefficient matrix $\mathbf{K}$ are known and constants.

A3. The biomass growth rates, φ_1, φ_2, and φ_3, are considered as unknown, time-varying parameters.

For the bioprocess, described by the system (4), (5) and under the assumptions A1÷A3, the following problem is considered: estimation of the biomass growth rates φ_1, φ_2, and φ_3, (respectively the specific biomass growth rates μ_1, μ_2, and μ_3) as

well as biomass concentration X by two observers using on-line measurements of S and A.

The model (5) can be presented in the form of the following two submodels:

I submodel, describing oxidative-fermentative state of the process:

if $q_s > q_{scr}$

$$\begin{aligned} \frac{dS}{dt} &= -k_1\varphi_1 - k_2\varphi_2 - DS + DS_{in} \\ \frac{dA}{dt} &= k_3\varphi_2 - DA \\ \frac{dX}{dt} &= \varphi_1 + \varphi_2 - mX - DX \end{aligned} \tag{6}$$

II submodel, describing oxidative state of the process:
if $q_s \le q_{scr}$

$$\begin{aligned} \frac{dS}{dt} &= -k_1\varphi_1 - DS + DS_{in} \\ \frac{dA}{dt} &= -k_4\varphi_3 - DA \\ \frac{dX}{dt} &= \varphi_1 + \varphi_3 - mX - DX \end{aligned} \tag{7}$$

where q_s is the glucose consumption rate and q_{scr} is its critical value, above which acetate is produced. m represents the maintenance coefficient, which is considered constant for the present study.

The above two submodels underlie of the proposed estimation algorithms of the biomass growth rates φ_1, φ_2, and φ_3, (respectively the specific biomass growth rates μ_1, μ_2, and μ_3) and biomass concentration X.

III. *Estimation Algorithms*

III.1 Estimator and Observer Design for the Oxidative-Fermentative Stage of the Process

Estimator of the growth rate φ_2; We assumed that:
A4. Noisy measurements A_m and S_m are available on-line:

$$A_m = A + \varepsilon_1; \qquad S_m = S + \varepsilon_2,$$

where ε_1 and ε_2 are measurement noises.

The following observer-based estimator of φ_2 is proposed using the dynamical equation of A concentration from the model (6):

$$\begin{aligned} \frac{d\hat{A}}{dt} &= -DA_m + k_3\hat{\varphi}_2 + C_{1a}(A_m - \hat{A}), \\ \frac{d\hat{\varphi}_2}{dt} &= C_{2a}(A_m - \hat{A}) \end{aligned} \tag{8}$$

where C_{1a}, C_{2a} are estimator parameters.

Stability Analysis; Consider the error system associated to the observer (8):

$$\frac{dx}{dt} = Ax + u \tag{9}$$

$$x = \begin{vmatrix} \tilde{A} \\ \tilde{\varphi}_2 \end{vmatrix} = \begin{vmatrix} A_m - \hat{A} \\ \varphi_2 - \hat{\varphi}_2 \end{vmatrix}; \quad A = \begin{vmatrix} -C_{1a} & k_3 \\ -C_{2a} & 0 \end{vmatrix};$$

$$u = \begin{vmatrix} -D\varepsilon_1 - C_{1a}\varepsilon_1 \\ -C_{2a}\varepsilon_1 + \frac{d\varphi_2}{dt} \end{vmatrix},$$

where x is the error vector, u is the input vector of the error system and A is the matrix of the error system. The C_{1a}, C_{2a} values have to be chosen such that matrix A remains stable, i.e. $C_{1a} > 0$ and $C_{2a} > 0$.

Observer of X and estimator of the growth rate φ_1: A "software sensor" is derived to estimate X and the growth rate φ_1, based on the dynamical equations of X and S (model (6)), considering the φ_2 estimates, obtained by the observer-based estimator (8) as on-line measurements. Since X is not observable from the S measurements, according the dynamical model (6), the following auxiliary parameter is defined, similarly to the algorithms in [2], [6]:

$$\alpha = X - \varphi_1, \tag{10}$$

Substituting φ_1 from (10) in the dynamical equations of S and X (6), the following observer of X is derived:

$$\begin{aligned} \frac{d\hat{S}}{dt} &= -DS_m + DS_{in} - k_2\varphi_{2m} - k_1(\hat{X} - \hat{\alpha}) + C_{1s}(S_m - \hat{S}) \\ \frac{d\hat{X}}{dt} &= \hat{X} - \hat{\alpha} + \varphi_{2m} - m\hat{X} - D\hat{X} + C_{2s}(S_m - \hat{S}) \\ \frac{d\hat{\alpha}}{dt} &= C_{3s}(S_m - \hat{S}) \end{aligned} \tag{11}$$

where C_{1s}, C_{2s} C_{3s} are observer parameters, φ_{2m} are the estimates of φ_2, obtained using the observer based estimator (8).

The estimates of φ_1 can be obtained using the model:

$$\hat{\varphi}_1 = \hat{X} - \hat{\alpha} \tag{12}$$

where $\hat{X}$ and $\hat{\alpha}$ are the estimates of X and α obtained from estimation algorithm (11). The estimates of the specific biomass growth rates μ_1 and μ_2 can be obtained on the basis of the estimates of φ_1 and φ_2 and the estimates of biomass concentration using the relationship between them (model (5)).

The stability of the observer (11) was proved.

III.2 Estimator and Observer Design for the Oxidative Stage of Process

Estimator of the growth rate φ_3: The following observer-based estimator of φ_3 is proposed using the dynamical equation of A concentration from the model (7):

$$\begin{aligned} \frac{d\hat{A}}{dt} &= -DA_m - k_4\hat{\varphi}_3 + C_{1a}(A_m - \hat{A}), \\ \frac{d\hat{\varphi}_3}{dt} &= C_{2a}(A_m - \hat{A}) \end{aligned} \tag{13}$$

where C_{1a}, C_{2a} are estimator parameters. Their values have to be chosen according the stability conditions.

Observer of X and estimator of the growth rate φ_1: The estimation of X and the growth rate φ_1 is carried out using an estimation algorithm, similarly to algorithm (11), based on the dynamical equations of X and S (submodel (7)). The φ_3 estimates, obtained by the observer-based estimator (13) are considered as on-line measurements. Similarly to the first submodel, the biomass concentration again is not observable from the S measurements, according the dynamical model (eq. 7). Again,

the parameter α is applied in the structure of the proposed estimation algorithm:

$$\alpha = X - \varphi_1$$

In this way, the following observer of X, estimating simultaneously X and α, is derived:

$$\begin{aligned} \frac{d\hat{S}}{dt} &= -DS_m + DS_{in} - k_1(\hat{X} - \hat{\alpha}) + C_{1s}(S_m - \hat{S}) \\ \frac{d\hat{X}}{dt} &= \hat{X} - \hat{\alpha} + \varphi_{3m} - m\hat{X} - D\hat{X} + C_{2s}(S_m - \hat{S}) \\ \frac{d\,\hat{\alpha}}{dt} &= C_{3s}(S_m - \hat{S}) \end{aligned} \qquad (14)$$

where C_{1s}, C_{2s} C_{3s} are observer parameters, φ_{3m} are the estimates of φ_3, obtained by the algorithm (13).

The stability is proved similarly to observer (11).

IV. *Results and Discussion*

The behaviour of the proposed estimation algorithms is investigated by simulations of a process model, derived on the basis of experimental data, obtained in the Fermentation Laboratory of Centro de Engenharia Biologica, Universidade do Minho, Portugal. This model can be presented by the submodels (6) and (7). The specific growth rates considered are:

$$q_s = q_{s\max}\frac{S}{K_s + S}\frac{K_i}{K_i + A}; \quad \mu_1 = q_s / k_1$$

$$\mu_2 = (q_s - q_{scr})/k_2, \quad \mu_3 = (\mu_{a\max} - \alpha_{ac}\mu_1)/k_4$$

where the values of the coefficients are: q_{smax}= 4.5 $g.g^{-1}.h^{-1}$, K_s=0.15 $g.kg^{-1}$, K_i=21.2 $g.kg^{-1}$, k_1=2.15, k_2=20, k_3=13.34, k_4=4.26, μ_{amax} =0.32 $g.g^{-1}.h^{-1}$, α_{ac}=0.905. The control input F_{in} values correspond to exponential growth of the biomass. The simulations are realized under different values of q_{scr}. The estimates of q_s change between 0.2 and 0.8. The values of design parameters C_{1s}, C_{2s} C_{3s}, C_{1a}, C_{2a} of the estimation algorithms are chosen using both stability conditions and a trial and error approach. The values of C_{1a} and C_{2a} are: C_{1a}= C_{2a}=1 for the observer based estimator (8) and: C_{1a}= 10 and C_{2a}= - $C_{1a}^2/4k_4$ for the observer based -estimator (13).

Figures 1 and 2 show the estimation results under q_{scr}= 0.2 and 0.5 respectively. The results of Figure 1 present the estimation of the specific biomass growth rates μ_1, μ_2, and μ_3 and the biomass and acetate concentrations for oxidative-fermentative stage of the process. As can be shown on Figure 1A, the development of specific growth rate μ_2 estimates reflects the development of acetate concentration: when the acetate concentration increases, μ_2 estimates has some positive values, when acetate concentration is almost constant - μ_2 estimates has smaller values. The results from Figures 2 present the estimation of the same parameters and variables for both oxidative fermentative and oxidative stages of the process. They show that when the acetate concentration increases, μ_2 estimates have positive values. The decreasing of the acetate concentration corresponds to positive values of μ_3 estimates. The μ_1 estimates tend to constant values, which are different ones depending on q_{scr} values. The estimates of biomass and acetate concentrations converge to the model values for both cases.

V. *Conclusion*

This paper presents a two- step approach for estimation of the specific biomass growth rates μ_1, μ_2, and μ_3 and the biomass concentrations X, of a fed-batch fermentation of recombinant *E. coli*.

The approach is based on a process model describing both oxidative fermentative and oxidative stages of the process, presented by a switch between two partial submodels. The results from the simulations show the good performance of the proposed estimation algorithms for both oxidative fermentative and oxidative stages of the process. The estimates of the three specific biomass growth rates are adequate ones with respect to the dynamics of biomass and acetate concentrations. The values of the tuning parameters were chosen using stability conditions and a trial and error approach. The future investigations of the proposed algorithms will be connected to their experimental validation, as well as the use of experimental data for the tuning of the observers.

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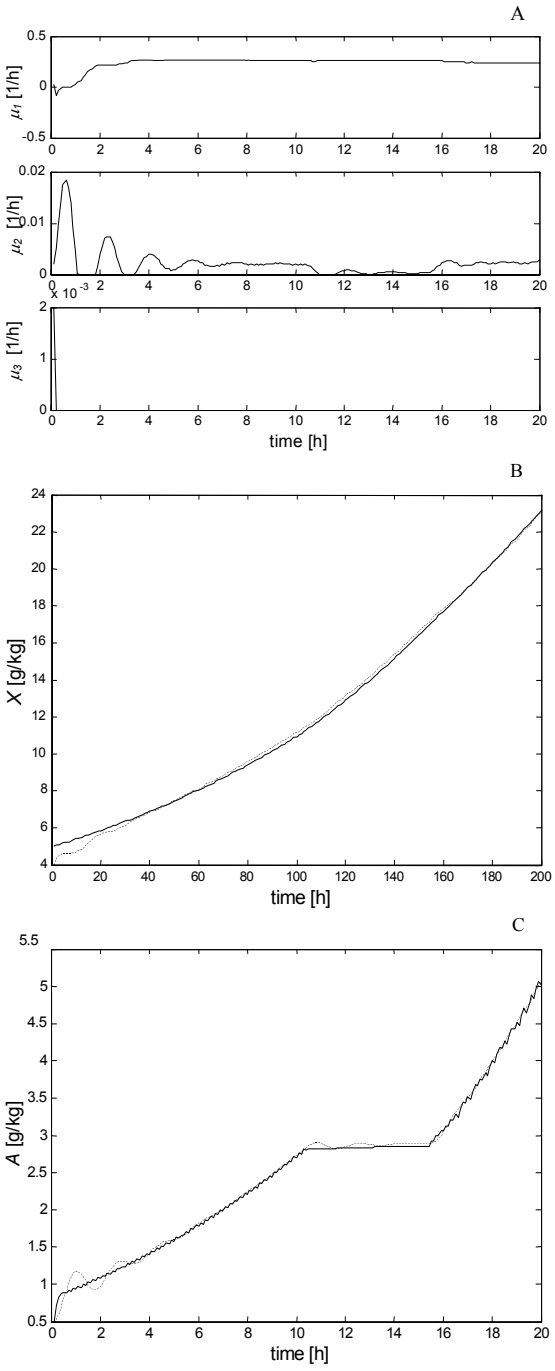

Figure1 Estimation of μ_1, μ_2, μ_3,, biomass X and acetate A concentrations: q_{cr}=0.2.

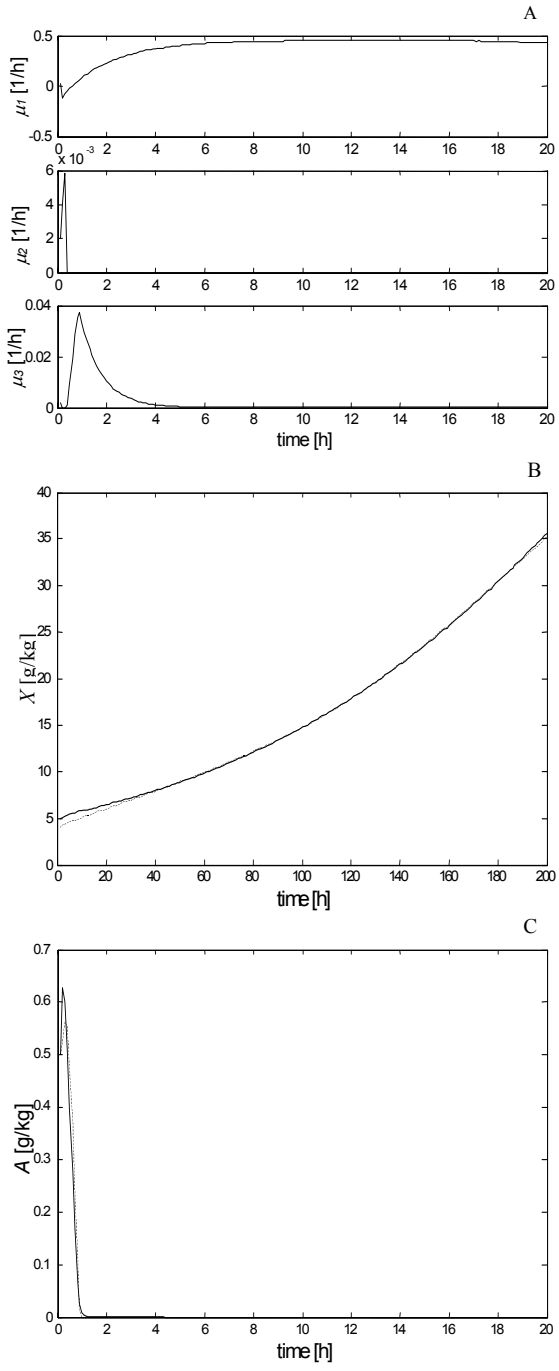

Figure 2 Estimation of μ_1, μ_2, and μ_3 and X and A concentrations: q_{cr}=0.5