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# Adaptive Biomass Observer in Fed-batch Cultivation of *Escherichia coli* on the Basis of On-line Measurements of Oxygen

Maya Ignatova, Velislava Lyubenova

*Department of Mehatronic Bio/Technological Systems, Institute of Robotics,*

*Bulgarian Academy of Sciences*

*Address: George Bonchev Str., Bl. 2, 1113 Sofia, Bulgaria*

*E-mails: maya.ign@gmail.com, v_lyubenova@ir.bas.bg*

## Abstract

A new adaptive asymptotic biomass observer for fed-batch *E. coli* growth on glucose is proposed. The observer works on the basis of on-line measurements of oxygen and stirrer speed only. The observation algorithm includes an on-line estimation procedure of yield coefficients on the basis of off-line measurements of biomass concentration.The preliminary investigations of the observer are realiased by simulations. The on-line measured experimental data is used as input information. The observation algorithm is verified by laboratory experiments of a recombinant *E. coli* strain.

## *Escherichia coli* as host organism for production of recombinant proteins.

*Escherichia coli* is a frequently used host organism for production of recombinant proteins. It has many advantages, such as being well characterized and support growth to high cell densities, but also drawbacks. One of the difficulties encountered in *Escherichia coli* cultivation is the formation of the metabolic by-product, acetate, *A,* in situations with excess glucose, *S,* 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 appropriate choice of cultivation conditions. In fed-batch cultures, where the additional glucose is fed during the cultivation, the fed rate can be manipulated to restrict formation of acetate. The feeding strategy is often complicated due to the lack of cheap and reliable on-line sensors. Furthermore, most strategies also require considerable process knowledge to work well. Indirect measurements can be obtained by combining the information from existing sensors using parameter and state estimation. As the process behavior is non-linear and time varying, it is common to propose adaptive control scheme that uses parameter and state estimated values. Therefore, the glucose concentration and the concentration of acetate in the reactor are considered to be zero during the fermentations. The concentration of the other main substrate, oxygen, *Co*, is kept at a constant value but the dynamics of the limiting substrates exists because of substrate feedings. Thereupon, the biomass grows on substrate feedings only. To address this problem, a design of biomass observer during the fed-batch fermentation where an optimal profile of glucose feeding is applied is proposed. That profile stabilizes the specific glucose rate at a critical value, $q_S^{crit}$.Therefore, the glucose concentration and the concentration of acetate in the reactor are considered to be zero during the fermentations. The concentration of the other main substrate, oxygen, is kept at a constant value but the dynamics of the limiting substrates exists because of substrate feedings Thereupon, the biomass grows on substrate feedings, $F_{in}S_f$ only.

## Biomass observers design

Biochemical models of aerobic growth of *Escherichia coli* on glucose presents the dynamics of main process variables, *X, S , A ,Co,* as functions of specific rates, $\mu$ , $q_s$, $q_o$, $(q_a^p - q_a^c)$ and substrate feed rates , $F_{in}.S_f$ in (1, 2, 3) . The reaction scheme of aerobic growth of *Escherichia coli* on glucose is well known and is presented in (4 ). Only the main process variables are included in the scheme (4). On the left-hand side, the substrates are appeared. On the right-hand side, the reaction products are presented. The reaction scheme (4) is a classical one and it consists of one main reaction $\varphi$ that represents the respiratory growth on glucose. Taking in to account the key process parameter, such as critical glucose uptake rate above, which the acetate occurs the scheme (4) could be presented with two reactions (5). For the considered process, an optimal profile of glucose feeding is applied. It guarantees a restriction of acetate production as well as a saturation of glucose uptake rate. The glucose and acetate concentrations are closed to zero in the reactor. The oxygen concentration is kept at a constant value and all transferred oxygen is used for degradation of fed glucose. The reaction scheme for this process has to be presented also by two reactions (6) . In the case $q_s < q_s^{crit}$ only the first reaction is activated but in the case $q_s \geq q_s^{crit}$ both reactions are activated.
In this case, the reaction $\varphi_1$, is constant with specific uptake rate $q_S^{crit}$ and the second, $\varphi_2$, represents the specific reaction $(q_S - q_S^{crit})$ See (3).
Depending of the value of specific glucose uptake rate, two different operational models is derived (7, 7a, 8, 8a) and two different biomass observers is designed (9 and 10).

**Biochemical model**

$$\frac{dV}{dt} = F_{in}$$
$$\frac{d(S.V)}{dt} = -q_S(X.V) + F_{in}S_f$$
$$\frac{d(X.V)}{dt} = \mu(X.V)$$
$$\frac{d(A.V)}{dt} = (q_a^p - q_a^c)(X.V)$$
$$\frac{d(C_O V)}{dt} = -q_O(X.V) + K_{La}(N).V.(C_O^* - C_O)$$

where

$$q_S = q_S^{\max}\frac{S}{k_S + S} \quad (1)$$

**Biochemical model** When $q_S < q_S^{crit}$

$$\mu = q_S Y_{SX}^{oxid}$$
$$q_a^p = 0$$
$$q_a^c = \min\left(\frac{q_a^{c.\max} A}{k_a + A}, \frac{q_O^{\max} - q_S Y_{OG}}{Y_{OA}}\right)$$
$$q_O = q_S Y_{OG} \quad (2)$$

**Biochemical model** When $q_S \geq q_S^{crit}$

$$\mu = q_S^{crit} Y_{SX}^{oxid} + (q_S - q_S^{crit}) Y_{SX}^{ferm}$$
$$q_a^p = (q_S - q_S^{crit}) Y_{S.A}$$
$$q_a^c = 0$$
$$q_O = q_S^{crit} Y_{OG} \quad (3)$$

**Reaction schemes**

$$S + O_2 + A \xrightarrow{\varphi} X + CO_2 + A \quad (4)$$
$$S + O_2 + A \xrightarrow{\varphi_1} X \quad (5)$$
$$S + O_2 \xrightarrow{\varphi_2} X + A$$
$$S + O_2 \xrightarrow{\varphi_1} X$$
$$S + O_2 \xrightarrow{\varphi_2} X \quad (6)$$

**Operational model** when $q_S < q_S^{crit}$

$$\dot{V} = F_{in}$$
$$\begin{bmatrix} S.V \\ X.V \\ C_O V \end{bmatrix} = \begin{bmatrix} -1 \\ k_1 \\ k_3 \end{bmatrix} \varphi_1 + \begin{bmatrix} F_{in}S_f \\ 0 \\ Q_{in}V \end{bmatrix} \quad (7)$$

$q_S \geq q_S^{crit}$

$$\begin{bmatrix} S.V \\ X.V \\ C_O V \\ OUR.V \end{bmatrix} = \begin{bmatrix} -1 & -1 \\ k_1 & k_2 \\ k_3 & 0 \\ k_4 & k_5 \end{bmatrix} \begin{bmatrix} \varphi_1 \\ \varphi_2 \end{bmatrix} + \begin{bmatrix} F_{in}S_f \\ 0 \\ Q_{in}V \\ 0 \end{bmatrix} \quad (8)$$

**Operational model** when $q_S < q_S^{crit}$

$$\frac{dV}{dt} = F_{in}; \quad (7a)$$
$$\frac{d(S.V)}{dt} = -\varphi_1 + F_{in}S_f;$$
$$\frac{d(X.V)}{dt} = k_1\varphi_1;$$
$$\frac{d(C_o V)}{dt} = -k_3\varphi_1 + Q_{in}.V;$$

where

$$k_1 = Y_{SX}^{oxid},$$
$$k_3 = Y_{OG};$$
$$\varphi_1 = q_S V.X;$$
$$Q_{in}.V = K_{La}(N)(C_O^* - C_O).V;$$

**Operational model** when $q_S \geq q_S^{crit}$

$$\frac{dV}{dt} = F_{in}; \quad (8a)$$
$$\frac{d(S.V)}{dt} = -\varphi_1 - \varphi_2 + F_{in}S_f;$$
$$\frac{d(X.V)}{dt} = k_1\varphi_1 + k_2\varphi_2;$$
$$\frac{d(C_o V)}{dt} = -k_3\varphi_1 + Q_{in}.V;$$
$$\frac{d(OUR.V)}{dt} = k_4\varphi_1 + k_5\varphi_2.$$

where

$$k_1 = Y_{SX}^{oxid},$$
$$k_2 = Y_{SX}^{fe}$$
$$k_3 = Y_{OG};$$
$$k_4 = k_1 k_3 q_S^{crit}$$
$$k_5 = k_2 k_3 q_S^{crit}$$
$$\varphi_1 = q_S^{crit} V.X;$$
$$\varphi_2 = (q_S - q_S^{crit})V.X;$$
$$OURV = k_3 q_S^{crit} X.V;$$
$$Q_{in}V = K_{La}(N)(C_O^* - C_O).V;$$

The operational model (7, 8) consists of two main parts the process kinetics and the transport dynamics In the considered case, the process kinetics is unknown and the transport dynamics is known and must be used. A transformation of the models (7, 8) is made in such a way that the dynamics of the process is presented with known information only – measured variables - oxygen concentration, $C_O$ , oxygen uptake rate, *OUR.V* and transport dynamics (terms $Q_{in}V$ and $F_{in}S_f$ ). The model transformation is made applying the Z-transformation.

For the considered case two auxiliary variables are derived $\boldsymbol{Z_1}$ and $\boldsymbol{Z_2}$ . The auxiliary variable $\boldsymbol{Z_1}$ depends of glucose concentration that is equal to zero because of the optimal feeding profile. The auxiliary variable $Z_2$ depends of biomass concentration can be presented as follows: $\boldsymbol{Z_2 = -k_1 Z_1 + X.V.}$ For the model (7a) the biomass observer is presented as follows

$$X = (Z_2 + k_1 Z_1)/V \quad (9)$$

For the model (8a) the biomass observer is presented with (10)

$$\hat{X} = \left(Z_2 + \frac{k_1 q_S^{crit} - k_1}{k_2 q_S^{crit}}\left(Z_1 + \frac{1}{k_3}OURV\right) + \frac{k_1 q_S^{crit} - k_2}{k_2 q_S^{crit}}\left(Z_1 + \frac{1}{k_3}C_o.V\right)\right)/V$$

## Results

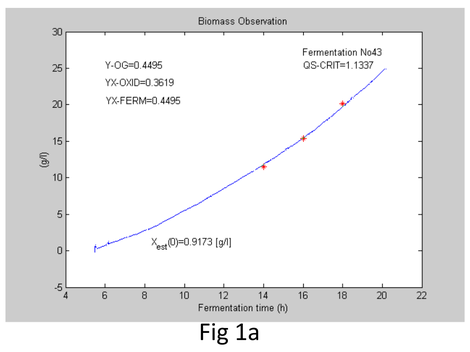


Fig 1a

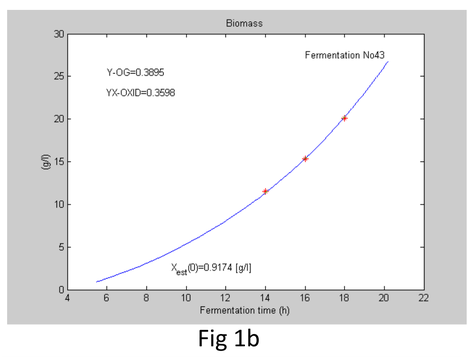


Fig 1b

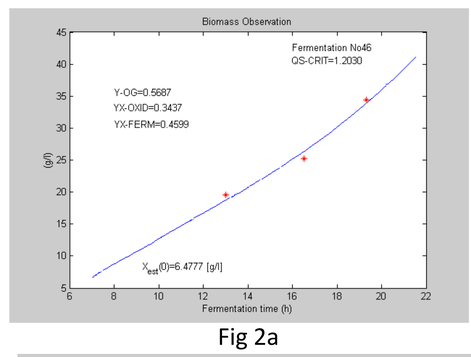


Fig 2a

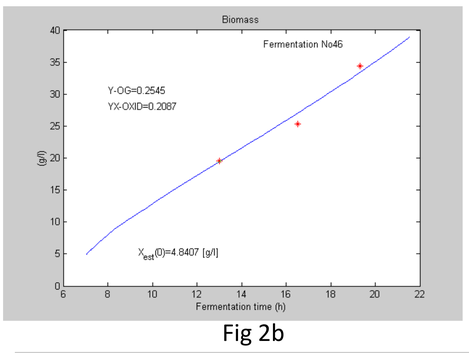


Fig 2b

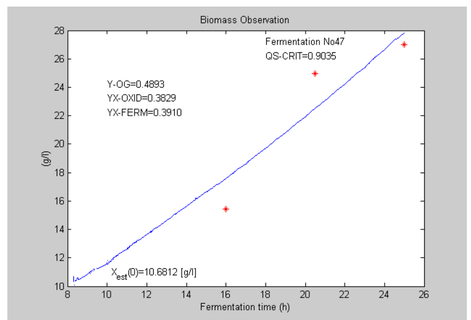


Fig. 3a

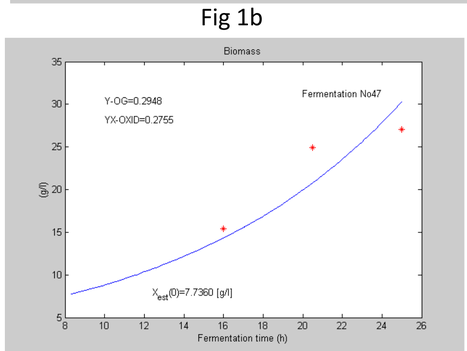


Fig. 3b

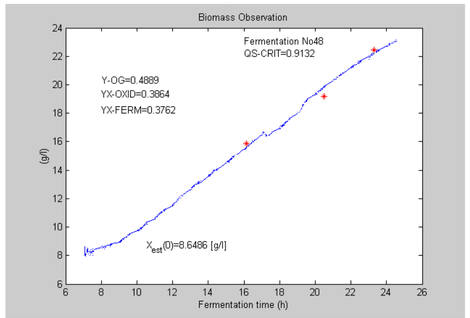


Fig. 4a

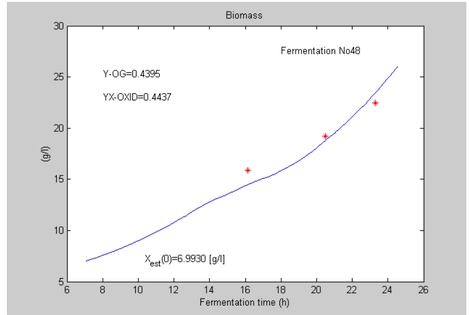


Fig. 4b

## Discussions

The experimental data of four fermentations of *E.coli* strain *Ifn* are used for simulation investigations of the proposed observers. The results are shown in figures. The figures with subscripts 'a' present the results where the algorithm (10) is applied. The figures with subscripts 'b' show the results where algorithm (9) is applied. The observations are presented with lines, and the measured points of biomass with stars. Comparing the results obtained up to 22 (or 26) hours of fermentation in sub-figures 2a and 3a, it was found that a fed-batch control (3a) achieves a higher concentration of the target product compared to the continuous control (2a). It should be noted that the fed- batch control process must be stopped when the volume reaches the maximum working volume (80 l), while during continuous mode the process may take longer. This does not give grounds for a definite conclusion under which cultivation regime will accumulate a larger amount of target product. In figures 1, the simulations with the experimental data of fermentation No 43 are sown. As can be seen, the observations by the algorithm (9) are better then the results obtained by the algorithm (10). Both algorithms produce satisfying results. Perhaps, the feeding profile during the experiment No 43 is a really optimal one. That profile stabilizes the process closed to the critical value of the glucose uptake rate. Therefore, the acetate production as well as the glucose concentration is closed to zero in the medium. On the figures 2,3 and 4, the simulations with fermentations No 46, 47 and 48 data are shown respectively. A comparison of the figures with subscripts 'a' and 'b' leads to the following conclusions. The algorithm (10) produces better biomass observations then the algorithm (9) where the critical specific glucose uptake rate is not taken in to account Perhaps during the fermentations No 46, 47 and 48, the feeding profiles are not optimal ones and some acetate is produced during the fermentations, but the acetate and the glucose concentrations are not measured in the medium during the experiments so these conclusions are the suspicions only. The simulation investigations lead to the following conclusions: The feeding strategy that is theoretically optimal one. The glucose feeding is calculated by an expression where the biomass concentration is estimated theoretically on the basis of its initial value. This value is one and the same for all fermentations, but the biomass growth is not the same during the experiments. Thereupon, the feeding profiles that were applied during the investigated fermentations were not always optimal ones. The algorithm (10) where the critical value of glucose uptake rate is included, produces better observations of biomass and that version of the algorithm has to be used for experimental investigations

## 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 in each time where the biomass measurements are received as additional off-line information. The better results could be obtained if those three points are being measured on the beginning of the fed-batch part of the cultivation. That off-line information would be good enough for biomass observer tuning.

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