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# ADAPTIVE CONTROL OF PROTEIN PRODUCTION BIOPROCESS WITH THREE PHYSIOLOGICAL STATES

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## INTRODUCTION

*A new adaptive linearizing control algorithm is proposed that stabilizes the carbon source concentration to a preset value. This algorithm is applied on recombinant protein production by Escherichia coli. A model for control of the investigated process is derived. The operating model includes three sub-models. Each of them describes a physiological state through which the process passes. Switching from one model to another depends on the sign of a key parameter obtained from the acetate measurements. A cascade scheme is derived from software sensors for the estimation of three biomass growth rates included in the structure of the proposed control algorithm. Simulation studies of the developed closed system have been carried out. The results with the impact of an open loop control system on the same object are compared.*

## SOFTWARE SENSORS,MARKER AND ESTIMATORS

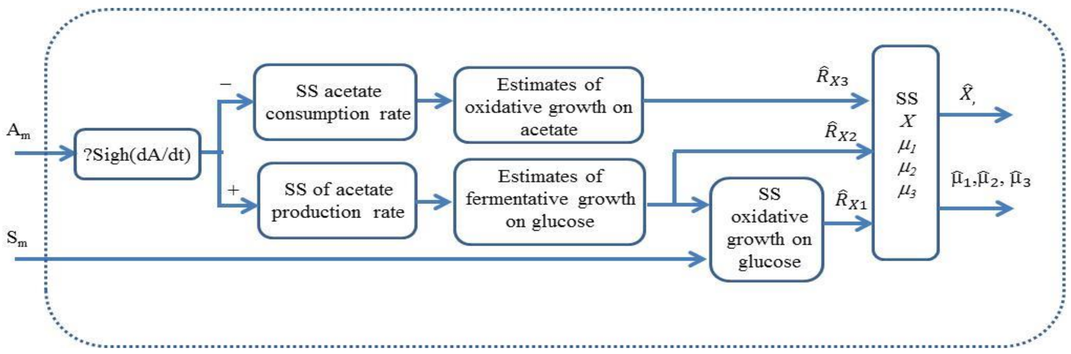


**Figure 1 Cascade structure of the software sensor for monitoring of two metabolic states**

$$\frac{d}{dt}\begin{bmatrix} X \\ S \\ A \end{bmatrix} = \begin{bmatrix} 1 & 1 \\ -k_1 & -k_2 \\ 0 & k_3 \end{bmatrix} \begin{bmatrix} \mu_1(t) \\ \mu_2(t) \end{bmatrix} X - D \begin{bmatrix} X \\ S \\ A \end{bmatrix} + \frac{F_{in,s}}{W} \begin{bmatrix} 0 \\ S_{in} \\ 0 \end{bmatrix} \quad (1)$$

$$\frac{d}{dt}\begin{bmatrix} X \\ S \\ A \end{bmatrix} = \begin{bmatrix} 1 & 1 \\ -k_1 & 0 \\ 0 & -k_4 \end{bmatrix} \begin{bmatrix} \mu_1(t) \\ \mu_3(t) \end{bmatrix} X - D \begin{bmatrix} X \\ S \\ A \end{bmatrix} + \frac{F_{in,s}}{W} \begin{bmatrix} 0 \\ S_{in} \\ 0 \end{bmatrix}$$

$$R_a = \frac{dA}{dt} + \frac{F_{in,S}}{W} A \quad (2)$$

*Ra > 0* *oxidative-fermentative growth on glucose*
*Ra = 0* *there is no acetate in the medium*
*Ra < 0* *oxidative growth on acetate*

### Oxidative growth on glucose

$$\frac{d\hat{S}}{dt} = -k_1\hat{R}_{X1} - k_2\hat{R}_{X2} - DS_m + \frac{F_{in,S}}{W}S_{in} + w_3(S_m - \hat{S})$$

$$\frac{d\hat{R}_{x1}}{dt} = w_4(S_m - \hat{S})$$

### Oxidative-fermentative growth on glucose

$$\frac{d\hat{A}}{dt} = \hat{R}_{ap} - DA_m + w_1(A_m - \hat{A})$$

$$\frac{d\hat{R}_{ap}}{dt} = w_2(A_m - \hat{A})$$

$$\hat{R}_{X2} = \hat{R}_{ap}/k_3$$

$$\frac{d\hat{S}}{dt} = -k_1\hat{R}_{X1} - k_2\hat{R}_{X2} - DS_m + \frac{F_{in,S}}{W}S_{in} + w_3(S_m - \hat{S})$$

$$\frac{d\hat{R}_{x1}}{dt} = w_4(S_m - \hat{S})$$

$$\frac{d\hat{X}}{dt} = \hat{R}_{X1} + \hat{R}_{X2} - D\hat{X}$$

$$\hat{\mu}_1 = \hat{R}_{X1}/\hat{X}$$

$$\hat{\mu}_2 = \hat{R}_{X2}/\hat{X}$$

$$\frac{d\hat{S}}{dt} = \hat{R}_S - DS + \frac{F_{in,S}}{W}S_{in} + w_5(S_m - \hat{S})$$

$$\frac{d\hat{R}_S}{dt} = w_6(S_m - \hat{S})$$

### Oxidative growth on acetate

$$\frac{d\hat{A}}{dt} = \hat{R}_{ac} - DA + w_5(A - \hat{A})$$

$$\frac{d\hat{R}_{ac}}{dt} = w_6(A - \hat{A})$$

$$\hat{R}_{X3} = -\hat{R}_{ac}/k_4$$

$$\frac{d\hat{X}}{dt} = \hat{R}_{X1} + \hat{R}_{X2} + \hat{R}_{X3} - D\hat{X}$$

$$\hat{\mu}_3 = \hat{R}_{X3}/\hat{X}$$

$$\hat{\mu}_1 = \hat{R}_{X1}/\hat{X}$$

**Table 1 Observer based estimators grouped, depending on the physiological state of the process**

Figure 1 shows the structure of the SS derived on the basis of operating models (1) and the new key parameter (2) for monitoring the three physiological states that are shown above. The SS structure is activated depending on the values of Ra, as shown above with the values indicating which of the three metabolic states the process is in. In Table 1 are shown the observer-based estimators of unknown kinetic parameters. In the first column are the estimators for the rate of oxidative growth, which are the only step of the software cascade that is switched on for the first physiological state. In second column (second physiological state) are the same estimators together with the estimators for the rate of acetate production, fermentative growth, concentration of the biomass and the specific rates, and the rate of glucose consummation in real time. The third column for the last state, shows the estimators for rate of acetate consummation, oxidative growth on acetate and the concentration of biomass and the specific rates in real time

## ADAPTIVE CONTROL AND RESULTS

| Parameters | $q_{s,max}$ | $K_s$ | $k_1$ | $k_2$ | $k_3$ | $k_4$ | $k_{os}$ | $q_{omax}$ | $K_{i,o}$ | $q_{ac,max}$ | $K_a$ | $K_{ia}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Value | 11.34 | 7.17 | 2.06 | 3.17 | 0.72 | 8.9 | 5.47 | 0.28 | 2.8 | 0.04 | 0.37 | 84.8 |

**Table 2 Kinetic parameters of the process**

On Table 2 are shown the values of the model parameters. An adaptive control is derived from the estimators shown above. Control based on the concentration of the glucose in the cultural medium is derived from the equations and it is shown in (3). For the values of S* was picked value of 0.01 and the concentration of glucose in the feed Sin is 250 g/l, chosen from expert's point of view.

$$F = \frac{W.(-\lambda(S^* - S_m) + k_1\hat{R}_{X1} + k_2\hat{R}_{X2} + k_4\hat{R}_{X3})}{S_{in} - S_m} \quad (3)$$

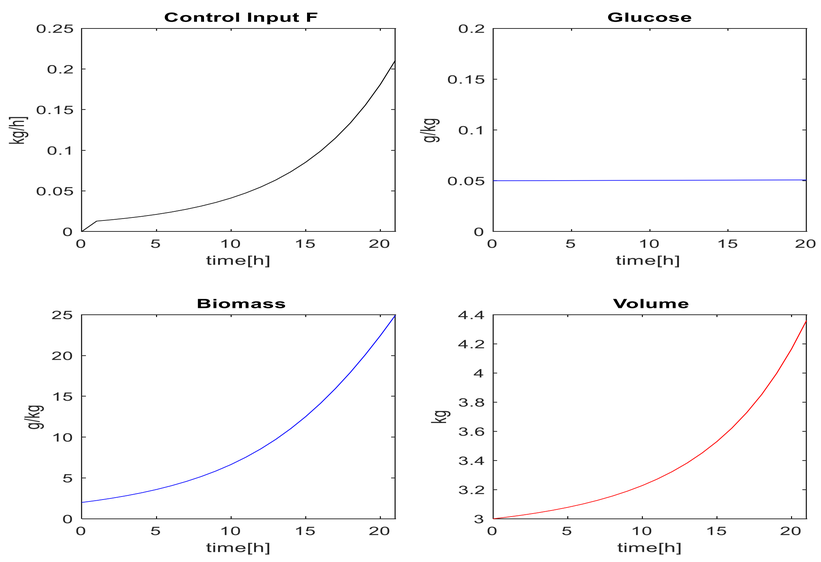


**Figure 2 Linearizing control algorithm investigation**

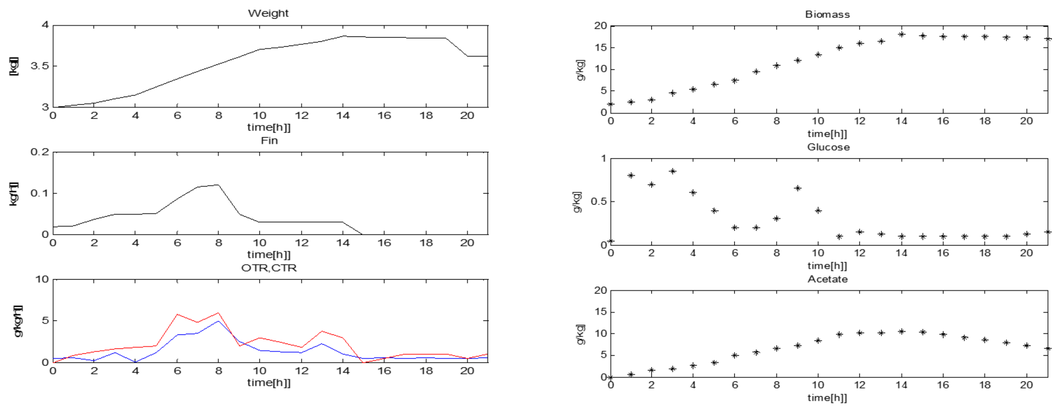


**Figure 3 Open loop control for the fermentation according the experimental data**

In figures 2 and 3, a comparison between two control algorithms can be seen. Figure 2 shows the results of the simulation investigations for the derived adaptive control. It can be noted that the control is turned on at the beginning of the process and up to 20 h the working volume of the bioreactor (5 kg) is not exceeded. In Figure 3, the results from open loop control are shown. The control is an exponential function precomputed and applied to the bioreactor.

Maintaining a constant value of S* can achieve more concentration of biomass which is the target product than in open loop control (Figure 3). It can be noted that the biomass concentration in the proposed control continues to grow until the end of the process, while in the open loop control, a retention and decline in biomass concentration after 14 h was observed.

This decrease may be due to the combination of factors, which are the increased biomass density during the fermentation process and the influence of acetate, which starts to be produced around the 2nd hour and being consummated at hour 14th. These factors inhibit the growth of biomass.

At the same time, the weight at the end of the process is higher in comparison with Figure 3, but still is in within the limits of the working volume of the bioreactor, which makes the efficiency of the control better.

## CONCLUSION

A closed loop control of fed-batch fermentation by Escherichia coli is proposed. The derived adaptive linearizing control aims to stabilize the glucose concentration at previous set value. It is proposed from expert' point of view. The process model is identified using the experimental data of batch part of fermentation. This model is used to design observers of unmeasured two biomass growth rates included in the structure of the proposed control algorithm. This solution leads to its adaptive properties.

A comparison between two control algorithms - open-loop and closed-loop control of the same object is realized. The adaptive properties of closed-loop systems are advantageous in controlling non-linear and non-stationary processes such as biotechnological ones. Further research have to be done to investigate the impact of various disturbances on monitoring and control as well as the choice of algorithm's tuning parameter before applying this method in laboratory conditions.

A decrease was observed in biomass concentration in the open loop control, most likely due to a combination of factors, which inhibit the growth of biomass and include increased biomass density during the fermentation process, the presence of acetate, which starts to be produced at 1 hour of the process and sharp decrease in glucose concentration at 13 hour, when the substrate feed had been stopped. The feed rate in the closed loop control creates a stabilized value of substrate in the medium, which lowers the effect of the inhibiting factors of the process.

## AKNOWLEDGEMENT

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