CONTROL OF CLASS BIOPROCESSES USING ON-LINE INFORMATION OF INTERMEDIATE METABOLITE PRODUCTION AND CONSUMPTION RATES

MAYA IGNATOVA[1], VELISLAVA LYUBENOVA

Bulgarian Academy of Sciences, Institute of Control and System Research, Bulgaria, Sofia

Abstract*:* A class of bioprocesses is defined where intermediate metabolite is produced in one reaction and then is used as substrate in other process reaction. New methods for adaptive control are proposed using estimators of intermediate metabolite production and consumption rates. An optimal tuning procedure for estimator's parameters is applied. As case study, poly-β-hydroxybutyric acid production by mixed culture is given. The simulation results demonstrate a tolerance of the control methods to uncertainty and to lack of measurements of the main process variables.

Keywords: Adaptive control, Reaction Rate Estimators, Fed-Batch, Continuous bioprocesses

INTRODUCTION

There exist a lot of mono culture bioprocesses where a metabolite is produced during one process reaction as intermediate product by biomass growth on the basis of a main substrate (usually carbon source) and then it is consumed as substrate for the growth of same biomass in the other process reaction. So, during one process there is production and consumption of metabolite that is considered as main process variable. Moreover, such a metabolite could be a target product for some processes. As examples: i) in well known recombinant protein production by fed-batch or continuous cultivation of *Saccharomyces cerevisiae* (or *E. coli*), during the fermentative growth of biomass on glucose, ethanol (and/or acetate) is produced and during the following phase, this product is used as substrate for oxidative growth of biomass on ethanol (and/or acetate); ii) the process of gluconic acid production by *Aspergillus niger,* the gluconic acid is produced at the

[1] Corresponding authors: Maya Ignatova, Velislava Lyubenova, Bulgarian Academy of Sciences, Institute of Control and System Research, Bulgaria, Sofia 1113, P.O.Box 79, e-mails: *ignatova@icsr.bas.bg* *lyubenova@icsr.bas.bg*

beginning of the process and when the main carbon source is exhausted, the gluconic acid is used for biomass growth. In general, the bioprocesses mentioned above could be summarised according to the role of the intermediate metabolite in the bioprocess: i) as main substrate for target product production: recombinant protein (Fredriksson, 2001, Ignatova et al.,2003, Jana et al.,2005, Koh et al., 1992, Li et al., 1998, Lubenova et al., 2003(1), Lyubenova et al., 2003 (2), Lyubenova et al., 2003(3)) etc. ii) as target product: ethanol (Altintas et al.,2002, Phisalaphong et al., 2006, Ülguen et al., 2003, Wang, et al., 2001); gluconic acid (Garsia et al., 2005, Ignatova et al., 2005, Ignatova et al., 2006, Ignatova et al., 2007) etc. Those bioprocesses are carried out as fed-batch or continuous. Usually, one of the problems of optimal process leading is the time of change of cultivation mode due to the lack of real life experiments reproducibility. In other words, the information about intermediate metabolite production and consumption rates could be used for dynamic recognitions of process physiological states.

Special interests provoke mixed culture processes (Patnaik, 2005). They are widely used in food industry (wine, beer, milk) as well as in wastewater treatment processes (Mazouni et al., 2004, Simeonov et al., 2001). As a rule, one microorganism produces an intermediate metabolite that is used as main substrate for other microorganism growth. The target process product depends of second biomass concentration in the reactor. Moreover, the growth of each microorganism needs different cultivation conditions (dissolved oxygen, pH, T^oC) that have to be changed for optimal control. In this way the growth of both biomasses will be stimulated separately So, software sensors for intermediate metabolite production and consumption rates could plays a key role in these cases. As an example, the microbial production of poly-β-hydroxybutyric acid (PHB) by mixed culture of *Lactobacillus delbrulckii* and *Ralstonia eutropha* is considered (Dias et al., 2005, Ganduri et al., 2005, Khanna et al., 2005, Lyubenova et al., 2006, Lyubenova et al., 2007, Patnaik, 2005, Tohyama et al., 2002) This process is sufficiently complicated and will be taken as example to demonstrate different new methods for adaptive control presented below.

An investigation related to control of fed-batch fermentation of mixed culture of *L. delbrulckii* and *R. eutropha* is carried out experimentally in (Tohyama et al., 2002). Two control outputs, glucose and lactate concentrations are measured on-line. Two control inputs, dissolved oxygen concentration (*DO*) and glucose feed rate, are used to maintain the control outputs at constant set values that are optimal from expert's point of view. The optimal value of

lactate is derived theoretically and it guarantees maximum growth of *R. eutropha*, and maximum final target product, (PHB). The lactate is produced by *L. delbrulckii*, on the basis of glucose and low value of *DO*. The produced lactate is used as carbon source for the *R. eutropha* growth at higher value of *DO*. The *DO* control is realized experimentally by bang-bang switching over low and high values, depending on lactate concentration in the reactor. The control of glucose feed rate is realized as impulses in the moments when the glucose is lower than the set value.

One of the disadvantages of the proposed in (Tohyama et al., 2002) control is that it is realized using measurements of lactate and glucose concentrations as outputs of two separate control loops which are interacted in practice. Moreover, the control is realized off-line on the basis of expert's experience.

Concentrations of glucose and lactate are integral variables, which generalize the growth of both microorganisms. Their kinetics has to be estimated and toward optimal control design.

The main purpose is synthesis of new methods for on-line adaptive control of PHB production. The measurements of glucose and lactate concentrations to be used for derivation of new kinetic parameters software sensors describing the growth of both microorganisms separately. The control schemes to be investigated by simulations where an unstructured model to be used as object and the software sensors to be designed on the basis of a control model.

UNSTRUCTURED PROCESS MODEL

A lot of experiments (Tohyama et al., 2002) are done to investigate the fermentation of mixed culture of *L. delbrulckii* and *R. eutropha*. Each experiment starts as mono batch aerobic fermentation of *L. delbrulckii* where glucose is the main carbon source. After 4 hours, *R. eutropha*, is inoculated and lactate that is produced by *L. delbrulcki* converts to PHB by *R. eutropha* in the oxygen and ammonium presence.

The following unstructured model is proposed in (Tohyama et al., 2002). It describes the dynamics of *L. delbrulckii* and *R. eutropha* based on mass balances with appropriate kinetic expression:

$$\dot{X}_1 = \mu_1(S, L, DO)X_1 - F.X_1/V; \quad (1a)$$

$$\dot{S} = -\nu_1(S, L, DO)X_1 - F(S_{in} - S)/V; \quad (1b)$$

$$\dot{L} = \sigma_1(S, L, DO)X_1 - \nu_2(S, L, DO)X_2 - F.L/V; \quad (1c)$$

$$\dot{X}_2 = \mu_2(L, DO, N)X_2 - F.X_2/V; \quad (1d)$$

$$\dot{N} = -\nu_3(L, DO, N)X_2 - F.N/V; \quad (1e)$$

$$\dot{PHB} = \sigma_2(N)X_2 - F.PHB/V; \quad (1f)$$

where X_1 is cell concentration of *L. delbrueckii*; X_2 – cell concentration of *R. eutropha*; S – glucose concentration; S_{in} – glucose concentration in the feed; V - broth volume; L - lactate concentration; N – ammonium, NH_3, concentration; PHB – PHB concentration; DO – dissolved oxygen concentration, μ_1 and μ_2 are the specific growth rates of *L. delbrueckii* and *R. eutropha* respectively; with: ν_1 - the specific glucose consumption rate; ν_2 - specific lactate consumption rate; ν_3 - specific NH_3 consumption rate; σ_1 - the specific lactate production rate; σ_2 - specific PHB production rate.
Parameter values of the unstructured model (1) are obtained in experimental way and they are given in (Tohyama et al., 2002). In Figure 1(a-f), the batch phase of the model is shown with points. In Figure 1d, the inoculation of *R. eutropha* at 4h of fermentation can be observed.

MODEL FOR CONTROL DERIVATION

According to the General Dynamical Model (GDM) Approach (Bastin et al., 1990), the model for control of a biotechnological process can be derived on the basis of a process reaction scheme. The mechanism of PHB production by mixed culture of *L. delbrulckii* and *R. eutropha* could be presented by the following reaction scheme:

$$\begin{aligned} S + DO &\xrightarrow{\varphi_1} X_1 + L \\ L + N + DO &\xrightarrow{\varphi_2} X_2 + PHB \\ L + DO &\xrightarrow[X_2]{\varphi_3} PHB \end{aligned} \quad (2)$$

The scheme (2) consists of three reactions - φ_1, φ_2 and φ_3. The first one represents growth associated production of lactate, *L*. The glucose, *S*, in the oxygen presence is converted to lactate by *L delbrulckii*, X_1. The second reaction represents growth associated production of PHB. The lactate in the oxygen and ammonium, *N*, presence is converted to PHB by *R. eutropha*, X_2. The third reaction represents non-growth associated production of PHB where the biomass plays simply the role of catalyst.
Following the rules proposed in (Bastin et al., 1990), the model for control is derived on the basis of reaction scheme (2) and it is presented as follows:

$$\dot{X}_1 = \varphi_1 - FX_1 / V \quad (3a)$$

$$\dot{S} = -k_1\varphi_1 - F(S - S_{in}) / V \quad (3b)$$

$$\dot{L} = k_2\varphi_1 - k_3\varphi_2 - k_4\varphi_3 - FL / V \quad (3c)$$

$$\dot{X}_2 = \varphi_2 - FX_2 / V \quad (3d)$$

$$\dot{N} = -k_5\varphi_2 - FN / V \quad (3e)$$

$$\dot{PHB} = k_6\varphi_2 + \varphi_3 - FPHB / V \quad (3f)$$

The model for control (3) has to describe the dynamics of the main process variable as well as the unstructured model (1). Comparing both models, they have different structures. Hence, the next step is model (3) parameters identification.

IDENTIFICATION OF THE MODEL FOR CONTROL

Identification of the model (3) parameters is realized using the batch phase of both process models applying an optimization procedure proposed in (Mazouni et al., 2004). The optimization criterion is the minimal mean square error between state variables of model (1) and model (3). The obtained optimal values of model (3) parameters are: k_1=9.1496, k_2=5.7282, k_3=4.2169, k_4=0.0715, k_5=0.4151, k_6= 0.0785

In Figure 1(a-f), simulations of model for control (3) are cross-validated with model (1) data. As can be seen in the figures, the model (3) (lines) describes the dynamics of the main process variables as well as the unstructured model (1) (points). A model with a structure (3) and parameters listed above could be used for process monitoring and control design.

DESIGN OF SUBSTRATE CONSUMPTION RATES ESTIMATORS

As only two variables are available (S and L) on-line, only two parameters could be estimated according necessary conditions for the process (3) observability (Bastin et al., 1990). We define two new kinetic parameters, ϕ_1 and ϕ_2. They are consumption rates of S and L given in (3b and 3c) respectively considered as unknown time-varying parameters.

$$\phi_1 = -k_1\varphi_1 \quad (4)$$

$$\phi_2 = -k_3\varphi_2 - k_4\varphi_3 \quad (5)$$

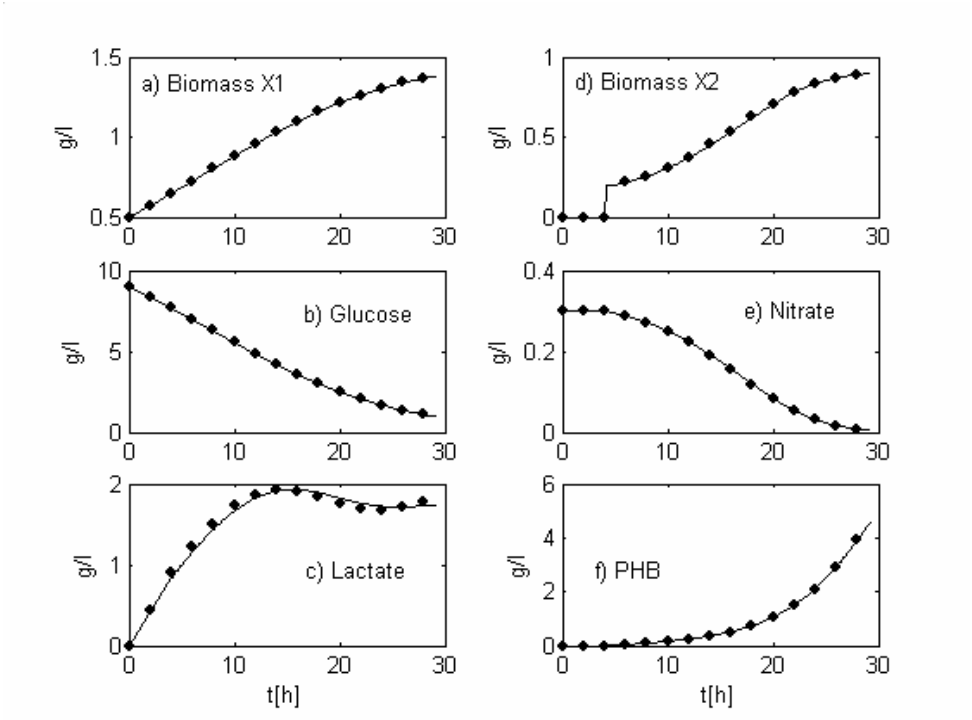

Figure 1. Batch phase of the model

The first step of the procedure proposed in (Lyubenova et al., 2007, Petre, 2002) is the estimation of consumption rate ϕ_1 (as well as estimation of reaction rate φ_1) using on-line measurements of glucose, S. The estimator of ϕ_1 is an observer-based estimator as follows:

$$\dot{\hat{S}} = \hat{\phi}_1 - F(S_m - S_{in})/V + \omega_1(S_m - \hat{S}) \tag{6}$$

$$\dot{\hat{\phi}}_1 = \gamma_1(S_m - \hat{S}) \tag{7}$$

where ω_1 and γ_1 are estimator design parameters, $S_m=S+\varepsilon$; ε is a measurement noise.

The design parameters of estimators (6, 7) are derived using an optimal tuning procedure, proposed in (Ignatova et al., 2007). In a result, the following expressions are obtained:

$$\omega_{1opt} = 2\zeta\sqrt{m_{11}/2m_{21}} \qquad \gamma_{1opt} = \omega_{1opt}^2/4\zeta^2 \tag{8}$$

where m_{11} is upper bound vector of time-derivative of ϕ_1; m_{21} - upper bound of additive noise of glucose measurement; ζ is a damping coefficient which value is fixed close to 1.

The estimates of reaction rate φ_1 can be calculated using the kinetic relationship (4):

$$\hat{\varphi}_1 = -\hat{\phi}_1 / k_1 \tag{9}$$

The next step is the estimation of ϕ_2 – consumption rate of lactate, using the on-line measurements of L and the estimates of φ_1 (9). The estimator of ϕ_2 is as follows:

$$\dot{\hat{L}} = k_3\hat{\phi}_1 + \hat{\phi}_2 - FL_m / V + \omega_2(L_m - \hat{L}) \tag{10}$$

$$\dot{\hat{\phi}}_2 = \gamma_2(L_m - \hat{L}) \tag{11}$$

where ω_2 and γ_2 are design parameters, $L_m=L+\varepsilon$; ε is an additive measurement noise.

Applying the procedure mentioned above, the following expressions are obtained:

$$\omega_{2opt} = 2\zeta\sqrt{m_{12} / 2m_{22}} \qquad \gamma_{2opt} = \omega_{2opt}^2 / 4\zeta^2 \tag{12}$$

where m_{12} is upper bound of the time-derivative of $\phi_2(t)$ and m_{22} - upper bound of ε.

ADAPTIVE CONTROL DESIGN

The aim at process control is to receive more target product PHB using an optimal expenditure of glucose. For this purpose, the lactate concentration has to be kept at an optimal value during the process. In such a way, the concentration of X_2 and the production of PHB will be increase. So, the optimal process control comes down to stabilization of lactate concentration at an optimal value in the reactor. Hence, software sensors of lactate production and consumption rates have to be designed.

The first term of the right hand side of (10) describes the lactate production rate and the second one – the lactate consumption rate. They could be defined as software sensors of those two kinetic parameters:

$$\hat{\Phi}_1 = k_5\hat{\phi}_1 \qquad \hat{\Phi}_2 = -\hat{\phi}_2 \tag{13}$$

Analyzing the models (1) and (3), the following conclusions could be formulated: i) parameter $\hat{\Phi}_1$ is proportional to X_1 growth rate and parameter

$\hat{\Phi}_2$ - to X_2 growth rate. Hence, each software sensor gives information for the growth rate of one microorganism. Using this information for control design, we could stimulate the growth of both microorganisms separately. ii) The growth of X_1 requires low DO value, but the growth of X_2 is stimulated by keeping a high DO value. The high and the low level of the dissolved oxygen are proposed by experts as 3 ppm and 0.5 ppm on the base of experiments (Tohyama et al., 2002). iii) High lactate concentration inhibits the X_2 growth as is shown in (Tohyama et al., 2002) where a Haldane model is proposed for this relationship. Taking in to account the Haldane relationship, an optimal value of L concentration in the reactor can be calculated theoretically by the expression: $L_{opt} = \sqrt{K_i K_P}$, iv) For optimal control of the considered process, L_{opt} has to be reached and kept during the fermentation, v) the growth of X_1 depends also on glucose concentration in the reactor.

We define the difference $\Delta = \hat{\Phi}_1 - \hat{\Phi}_2$ as a marker for stimulation of L production or consumption rate leading the process to the target L_{opt} . When Δ is negative, there is superiority to L consumption and the growth of X_1 has to be stimulated. When Δ is positive, there is superiority to L production and the growth of X_2 has to be provoked. This idea is designed as a control scheme shown in Figure 2. Two adaptive control algorithms are proposed. The first one is shown in Figure2 with solid lines only. For the second one the control scheme is extended with dished lines part. Both algorithms consist of two control input – glucose feed rate (F) and dissolved oxygen (DO) and two outputs glucose and lactate concentrations in the reactor. For simulations, the process is presented with unstructured model (1). The two outputs are corrupted by additive white noises (ε) and they are used for estimation of production (Φ_1) and consumption (Φ_2) rates of L. The difference Δ is used as marker to change the set value (DO^*) of dissolved oxygen controller from high to low level according the sign of Δ. Simultaneously, the information of software sensors (Φ_1 and Φ_2) is used for adaptive control design by glucose feed rate.

In cases where the Haldane model is not valid for the relationship between biomass growth and substrate, we can use other control strategy. For theses cases, the control scheme is extended (see Figure 2 dished lines extension). The measurements of glucose are used to design a closed loop for glucose concentration stabilization in the reactor. An adaptive linearizing control

algorithm is proposed for this purpose. The algorithm is derived on the basis of the control model (3) following rules proposed in (Bastin et al., 1990) and it is presented as follows:

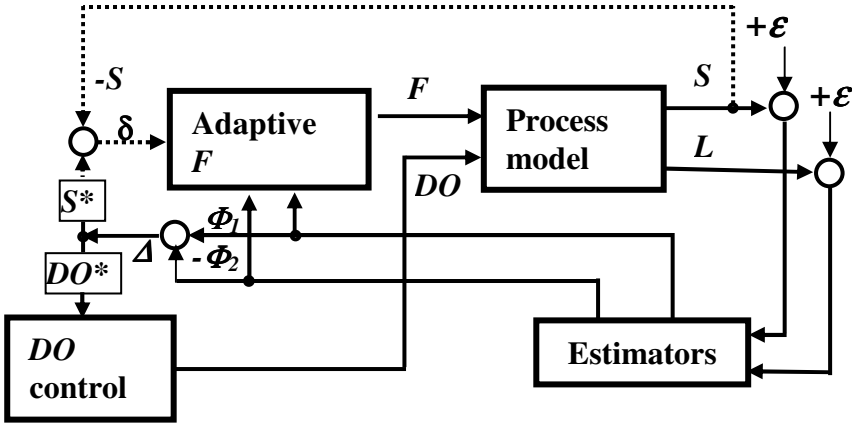

Figure 2. Control scheme

$$F = \frac{\lambda(S^* - S) + \hat{\Phi}_1 V}{S_{in} - S} \tag{14}$$

When the sign of Δ becomes negative for the first time, the mode of cultivation changes from batch to fed-batch. S^* could accept a value in the interval – the value of S at mode change and 0. Both algorithms are investigated by simulation of the control scheme and the results are discussed below.

SIMULATION INVESTIGATIONS

Simulations are carried out with Δ calculated from (6, 7), (10, 11) and (13). The design parameters γ and ω of estimators $\phi_1(t)$ and $\phi_2(t)$ are obtained by eqs. (8) and (12), respectively, where m_{11} and m_{12}, are set to 0.1. The white noise signals,ε, simulate measurement noises at standard deviation 5% of the mean S and L concentrations. The values of m_{21} and m_{22} are 0.07 and 0.119 respectively. Therefore, the optimal values of the design parameters are: ω_{1opt} = 1.685, γ_{1opt} = 0.724, ω_{2opt} = 0.83, γ_{2opt} = 0.175.

The simulation results are shown in Figs. 3 and 4. In Fig, 3a, a good tracking elapse of $\hat{\Phi}_1$ and $\hat{\Phi}_2$ can be observed. They follow the trends of "true" values obtained from model (1). In Figure 3b, a zoom of Δ is shown. In Figures 4a, b, the control outputs are shown.

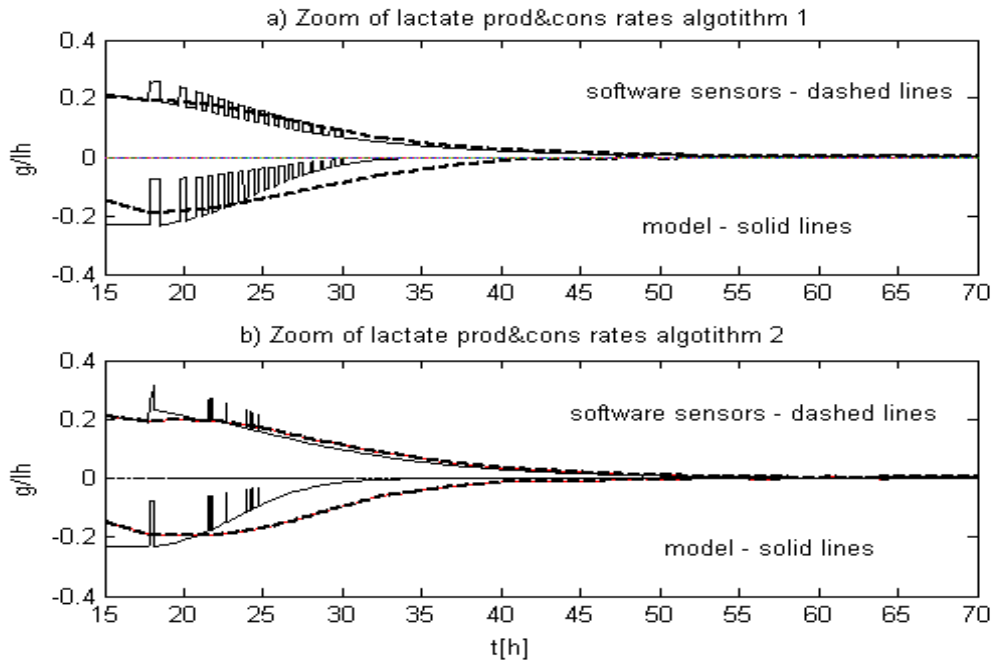

Figure 3. Simulation results

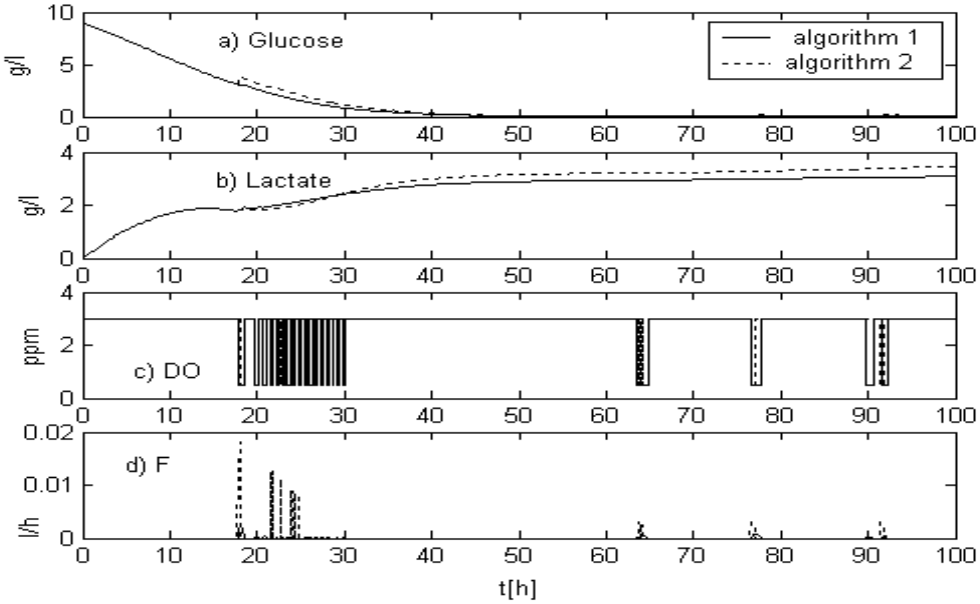

Figure 4. Control outputs

The switch-over the low *DO* level, as well as the switch on the glucose feeding, is realized, when Δ becomes negative, i.e. when is necessary to stimulate the growth of *L. delbrulckii*. For the control algorithm 1, the levels of the glucose feed impulses are determined by the dynamical equation of lactate concentration (eq. 10 without the last term), accepting zero dynamics, known kinetics (13) and optimal value of the lactate concentration, L_{opt} :

$$F_{opt} = (\hat{\Phi}_1 - \hat{\Phi}_2)V / L_{opt} \qquad (15)$$

For the algorithm 2, the levels of the glucose feed impulses are determined by the dynamical equation (14).

In the case of positive Δ, the *DO* switches on high level, and there is not *S* feed, i.e. the accumulated lactate is consummated by *R. eutropha*. In a result, the lactate concentration tends to its optimal value as is shown in Figure 4b. In figure 4a, the elapse of glucose is shown.

CONCLUSION

In this paper, two methods for two inputs-two outputs adaptive control of fed-batch process for PHB production by mixed culture are proposed. The process monitoring is enriched by new software sensors of lactate consumption and production rates. The difference between their values is considered as control marker and it is used for i) switch on batch to fed-batch phase automatically, ii) determination. of impulses duration of *DO* and glucose feeding. The levels of the glucose feed impulses are determined by two feeding profiles: (14) for the algorithm 2 and (15) for the algorithm 1.

As can be seen from algorithm (15), there are two parameters which have to be chosen - λ and S^*. Their values reflect on the control impulse numbers. Optimal values of those parameters have to be specified to avoid the chattering effect in real life experiments.

Simulation results show that both algorithms lead the process to the optimal value of lactate concentration. This guarantees maximum PHB productivity at the end of the process. The algorithms are derived on the basis of available (in the industry) on-line measurements. This gives reasons the control scheme in Figure 2 to be applied in real life experiments. Moreover, the same idea could be adapted for control of other processes where an intermediate metabolite is produced and consumed during the cultivation.

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