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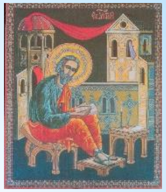
Почетен член на "Съвета на Европейската научна и културна общност”



New aspects in the development of the interactive system for education in modelling and control of bioprocesses

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Presentation Outline

1. General scheme of Open source system InSEMCoBio

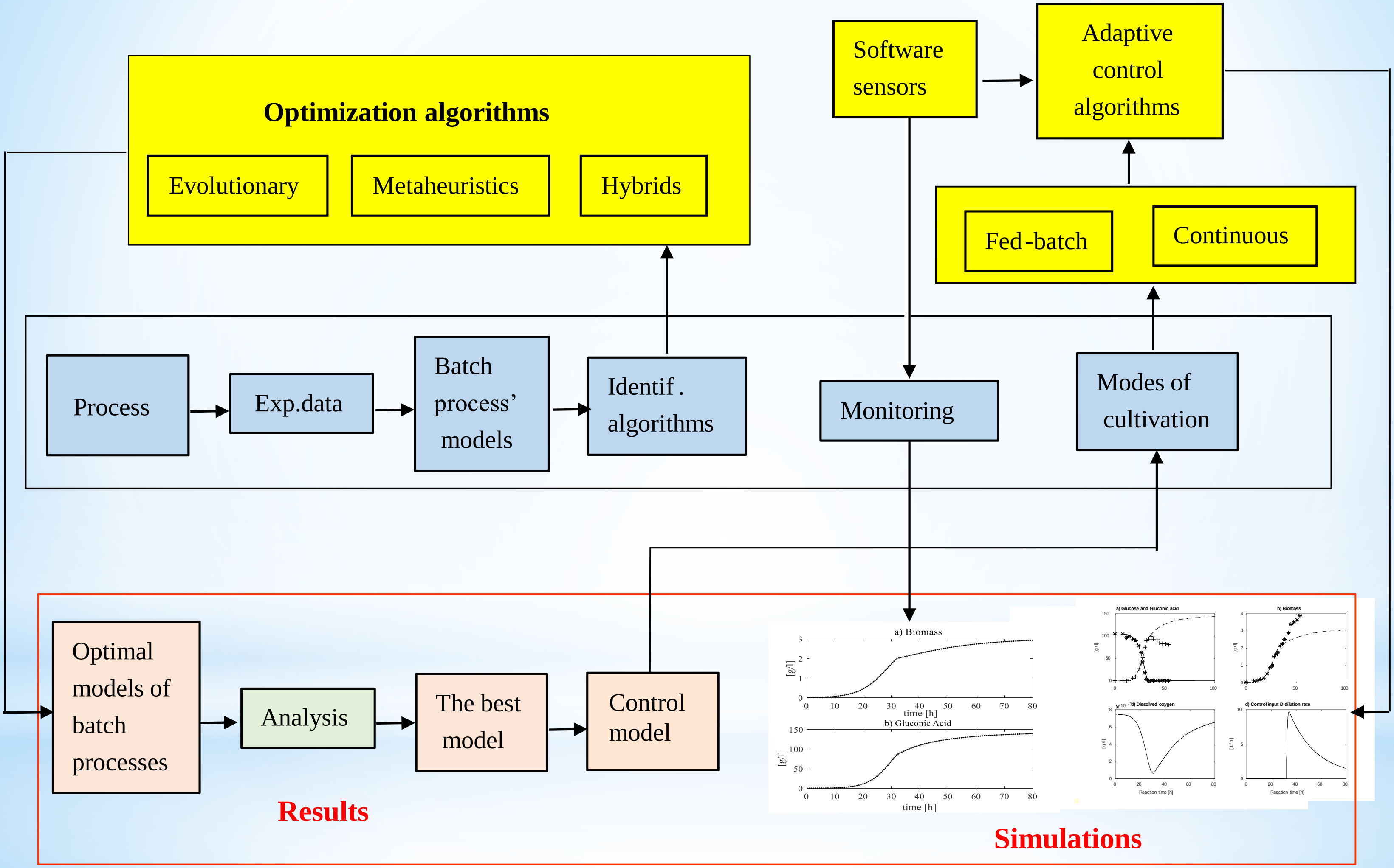
2. Evolutionary Algorithm Implemented in InSEMCoBio

3. Multistep Model for the case of extracellular production of bacterial phytase at fed-batch cultivation of *E. coli*

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

5. Adaptive Control of Protein Production Bioprocess with Three Physiological States

Open source system InSEMCoBio



Optimization algorithms

Evolutionary

Metaheuristics

Hybrids

Software sensors

Adaptive control algorithms

Fed-batch

Continuous

Process

Exp.data

Batch process' models

Identif. algorithms

Monitoring

Modes of cultivation

Optimal models of batch processes

Analysis

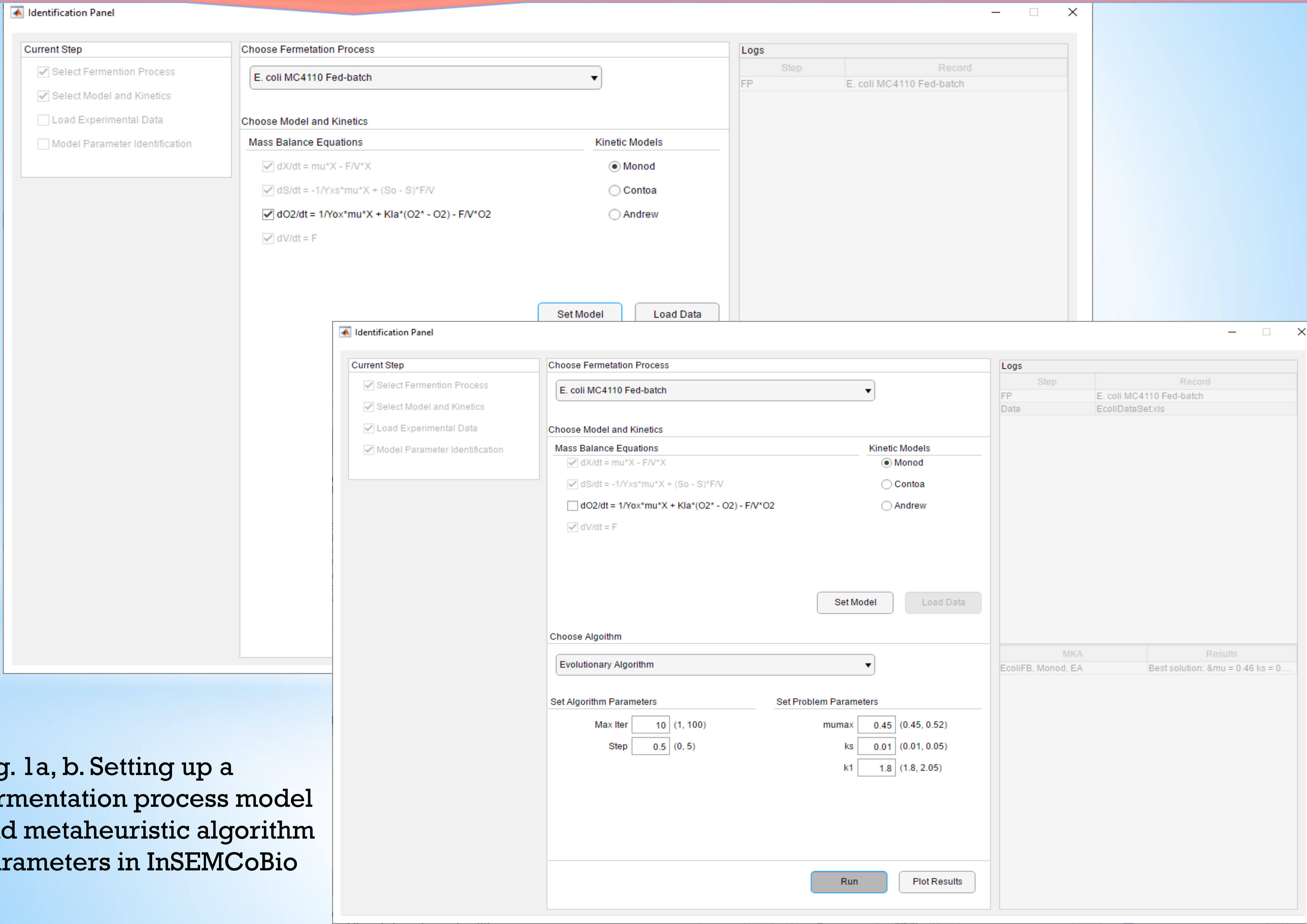
The best model

Control model

Results

Simulations

Evolutionary Algorithm Implemented in the Interactive System for Education in Modelling of Bioprocesses



Current Step

- ☑ Select Fermention Process
- ☑ Select Model and Kinetics
- ☐ Load Experimental Data
- ☐ Model Parameter Identification

Choose Fermetation Process: E. coli MC4110 Fed-batch

Choose Model and Kinetics

Mass Balance Equations

- ☑ dX/dt = mu*X - F/V*X
- ☑ dS/dt = -1/Yxs*mu*X + (So - S)*F/V
- ☑ dO2/dt = 1/Yox*mu*X + Kla*(O2* - O2) - F/V*O2
- ☑ dV/dt = F

Kinetic Models

- ● Monod
- ○ Contoa
- ○ Andrew

Logs

Step	Record
FP	E. coli MC4110 Fed-batch

Current Step

- ☑ Select Fermention Process
- ☑ Select Model and Kinetics
- ☑ Load Experimental Data
- ☑ Model Parameter Identification

Choose Fermetation Process: E. coli MC4110 Fed-batch

Choose Model and Kinetics

Mass Balance Equations

- ☑ dX/dt = mu*X - F/V*X
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- ☐ dO2/dt = 1/Yox*mu*X + Kla*(O2* - O2) - F/V*O2
- ☑ dV/dt = F

Kinetic Models

- ● Monod
- ○ Contoa
- ○ Andrew

Choose Algoithm: Evolutionary Algorithm

Set Algorithm Parameters

Max Iter 10 (1, 100)
Step 0.5 (0, 5)

Set Problem Parameters

mumax 0.45 (0.45, 0.52)
ks 0.01 (0.01, 0.05)
k1 1.8 (1.8, 2.05)

Logs

Step	Record
FP	E. coli MC4110 Fed-batch
Data	EcoliDataSet.xls

MKA	Results
EcoliFB, Monod, EA	Best solution: &mu = 0.46 ks = 0....

Fig. 1a, b. Setting up a fermentation process model and metaheuristic algorithm parameters in InSEMCoBio

Mathematical Model

The application of the general state space dynamical model to the fed-batch cultivation process of bacteria *E. coli* leads to the following nonlinear differential equation system :

$$\frac{dX}{dt} = \frac{\mu_{\max} S}{k_S + S} X + \frac{F}{V} X$$

$$\frac{dS}{dt} = -\frac{1}{Y_{XS}} \frac{\mu_{\max} S}{k_S + S} X + \frac{F}{V}\left(S_{in} - S\right)$$

$$\frac{dV}{dt} = F$$

X– the concentration of the biomass, [g/L],
S– the concentration of the substrate (glucose), [g/L];
F– the feeding rate, [L/h];
V– the volume of the bioreactor, [L];
S_{in}– the initial glucose concentration in the feeding solution, [g/L];
μ– the specific growth rate described by Monod kinetics, [1/h];
μ– the maximum growth rate, [1/h];
k_S– a saturation constant, [g/L];
Y_{XS}– a yield coefficient, [-].

Experimental Data and results

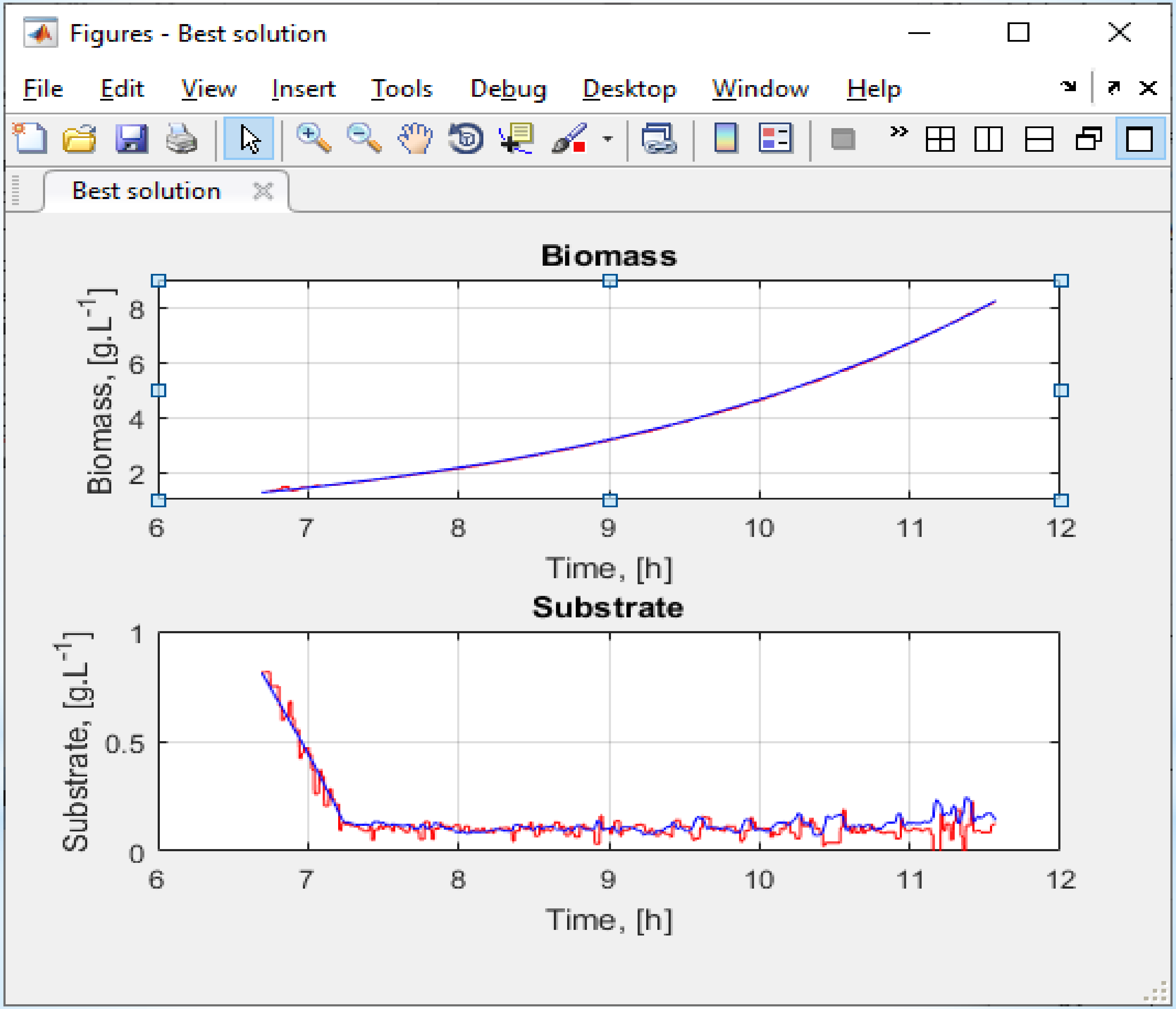


Fig. 1c Visualizing the results of the identification procedure in InSEMCoBio

Multistep Modeling of a Class Bioprocesses

Oxidative-fermentative growth model on glucose and oxidative on acetate

Oxidative-fermentative growth on glucose

$$R_{ac} > 0$$

$$\frac{d}{dt}\begin{vmatrix} X \\ S \\ A \\ P \end{vmatrix} = \begin{vmatrix} 1 & 1 \\ -k_1 & -k_2 \\ 0 & k_3 \\ k_5 & k_6 \end{vmatrix} \begin{vmatrix} \mu_1 \\ \mu_2 \end{vmatrix} - \frac{F}{V}\begin{vmatrix} X \\ S - S_{in} \\ A \\ P \end{vmatrix}$$

$$\mu_1 = q_{s,crit} / k_1$$

$$\mu_2 = (q_s - q_{s,crit}) / k_2$$

$$q_{s,crit} = \frac{q_{o,max}}{k_{os}} \frac{K_{i,o}}{K_{i,o} + A}$$

Marker

$$R_{ac} = \frac{dA}{dt} + \frac{F_{in,s}}{W} A$$

Oxidative growth on glucose and acetate

$$R_{ac} < 0$$

$$\frac{d}{dt}\begin{vmatrix} X \\ S \\ A \\ P \end{vmatrix} = \begin{vmatrix} 1 & 1 \\ -k_1 & 0 \\ 0 & -k_4 \\ k_5 & k_7 \end{vmatrix} \begin{vmatrix} \mu_1 \\ \mu_3 \end{vmatrix} - \frac{F}{V}\begin{vmatrix} X \\ S - S_{in} \\ A \\ P \end{vmatrix}$$

$$\mu_1 = q_{s,crit} / k_1$$

$$\mu_3 = q_{ac} / k_4$$

$$q_{ac} = q_{ac,max}\left(\frac{A}{K_A + A}\right)\left(\frac{K_{i,A}}{K_{i,A} + A}\right)$$

Fig. 2 Operational model of class bioprocesses

Experimental data of fed-batch phytase production

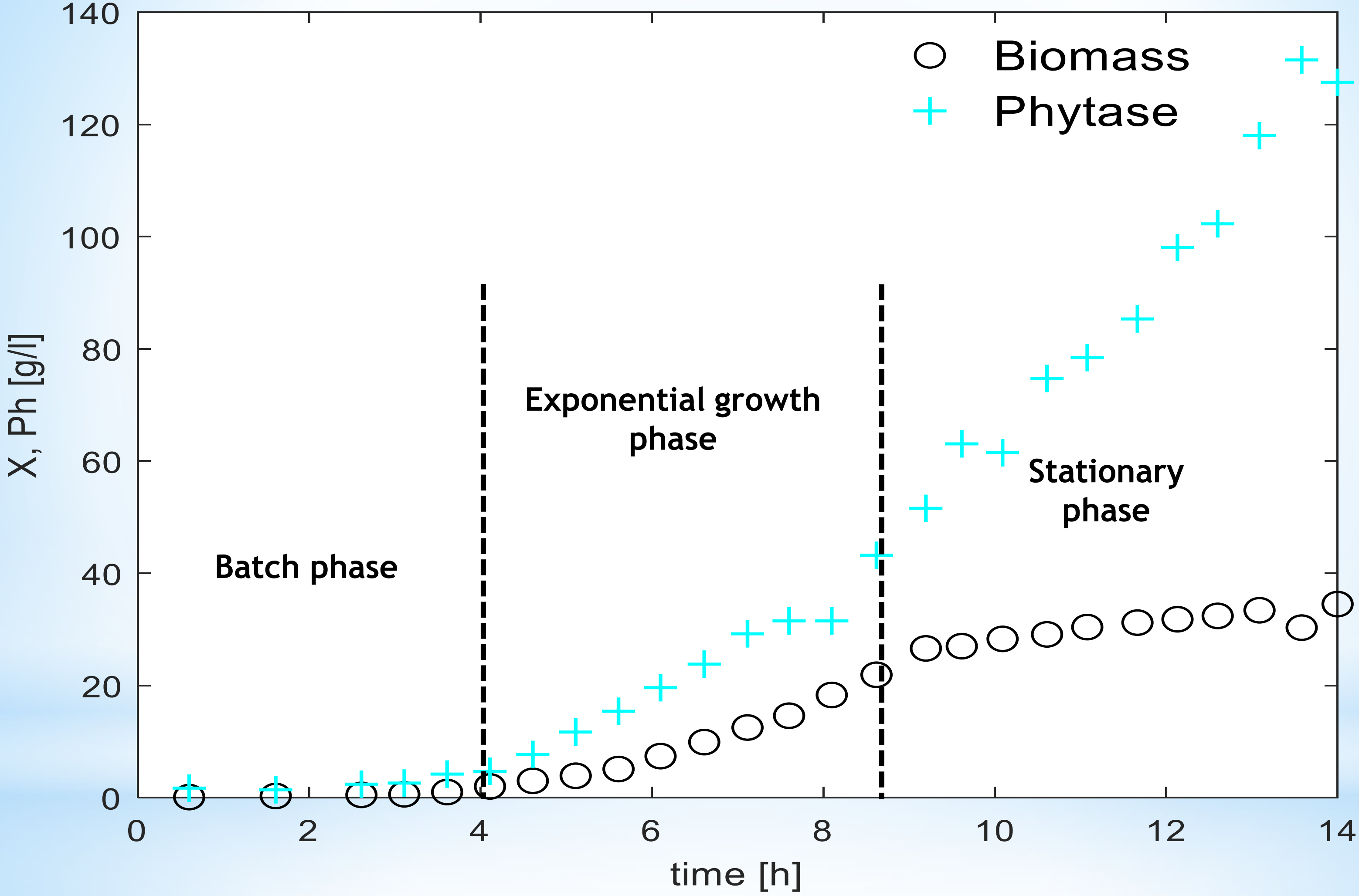


Fig. 3 Experimental data of biomass and phytase concentrations

Experimental data of fed-batch phytase production

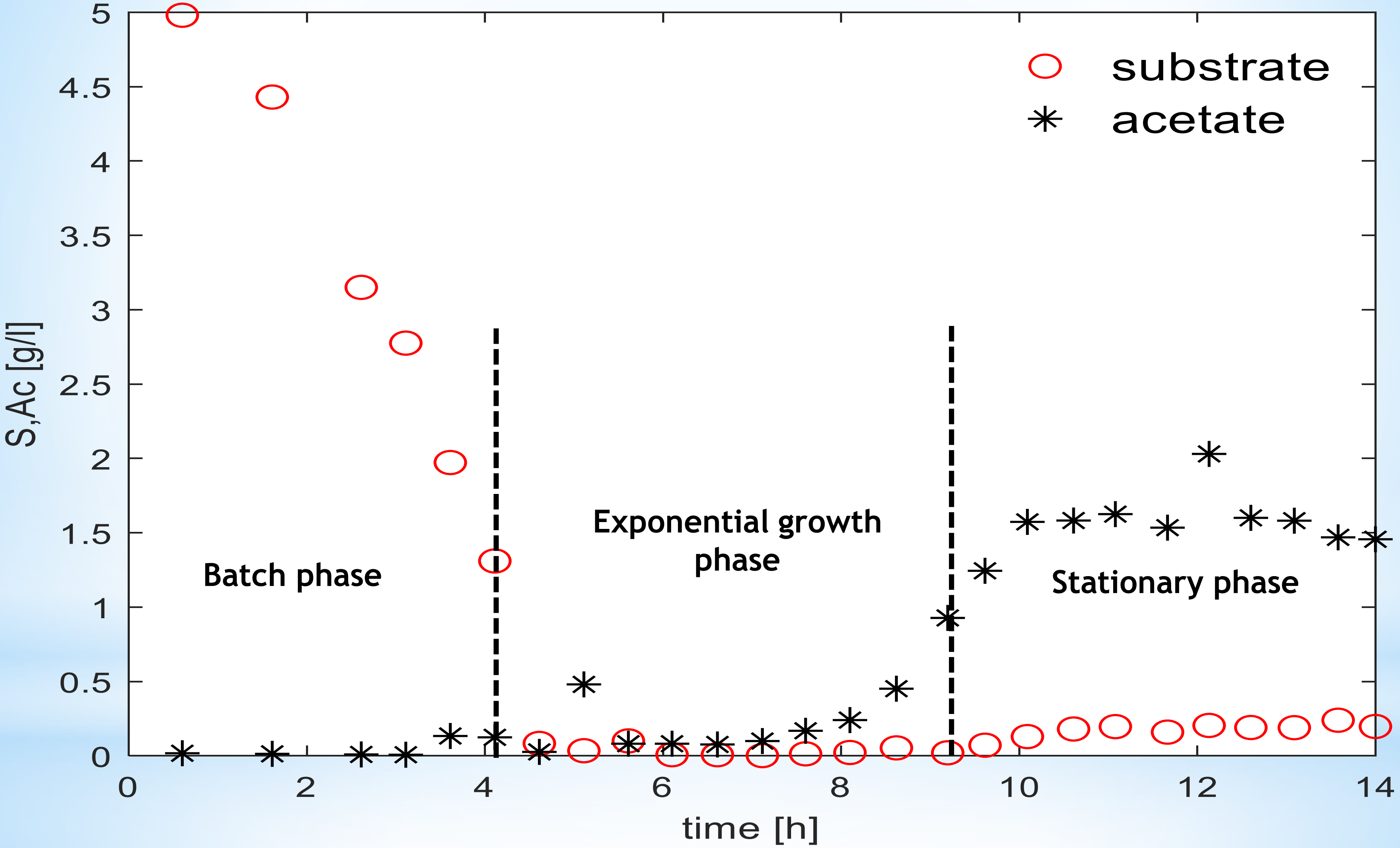


Fig. 4 Experimental data of substrate and acetate concentrations

Results

Table 1. Estimated kinetic parameters

	q_{smax}	k_s	k_{is}	q_{omax}	k_{os}	k_{io}	q_{acmax}	k_a	k_{ia}	k_1	k_2	k_3	k_4	k_5	k_6	k_7
1 phase	4.19	0.19	5.54	1.1	2.15	0.088	0.082	1.17	-	3.69	0.557	0.19	4.6	1.41	2.66	0.45
2 phase	34.24	0.79	1.83	0.469	2.53	0.197	0.143	0.97	0.246	2.08	2.167	0.05	4.1	2.88	1.52	0.5
3 phase	77.11	0.47	12.3	2.1	3.29	0.134	0.002	0.295	0.228	16.6	11.66	0.42	9.9	39.45	9.53	0.56

Simulation results

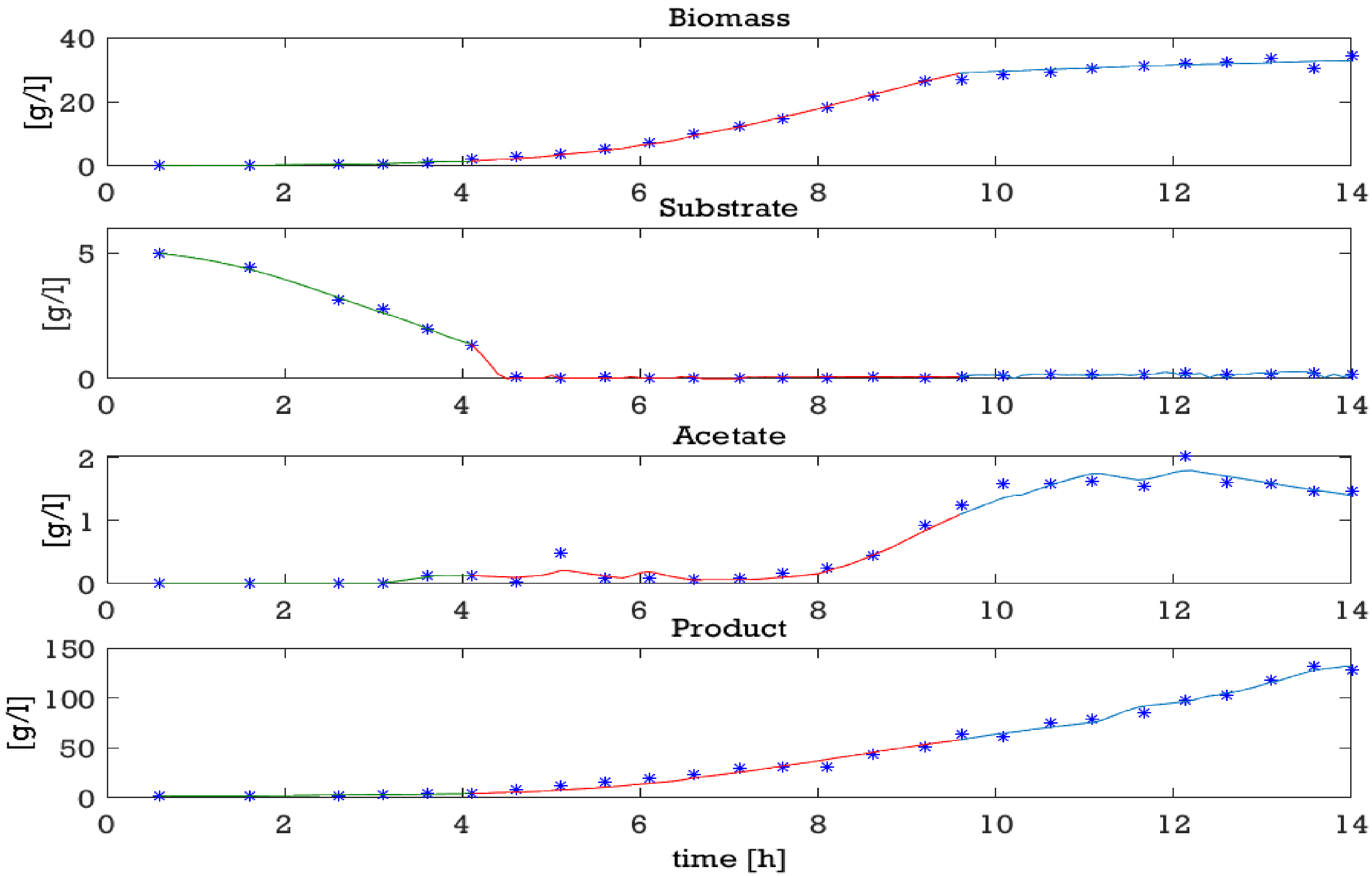


Fig. 5 Simulation results – models values of the biomass, substrate, acetate, and product concentrations are compared with experimental data for the three phases: batch phase – green lines, exponential growth phase – red lines, and stationary phase – blue lines.

Simulation results

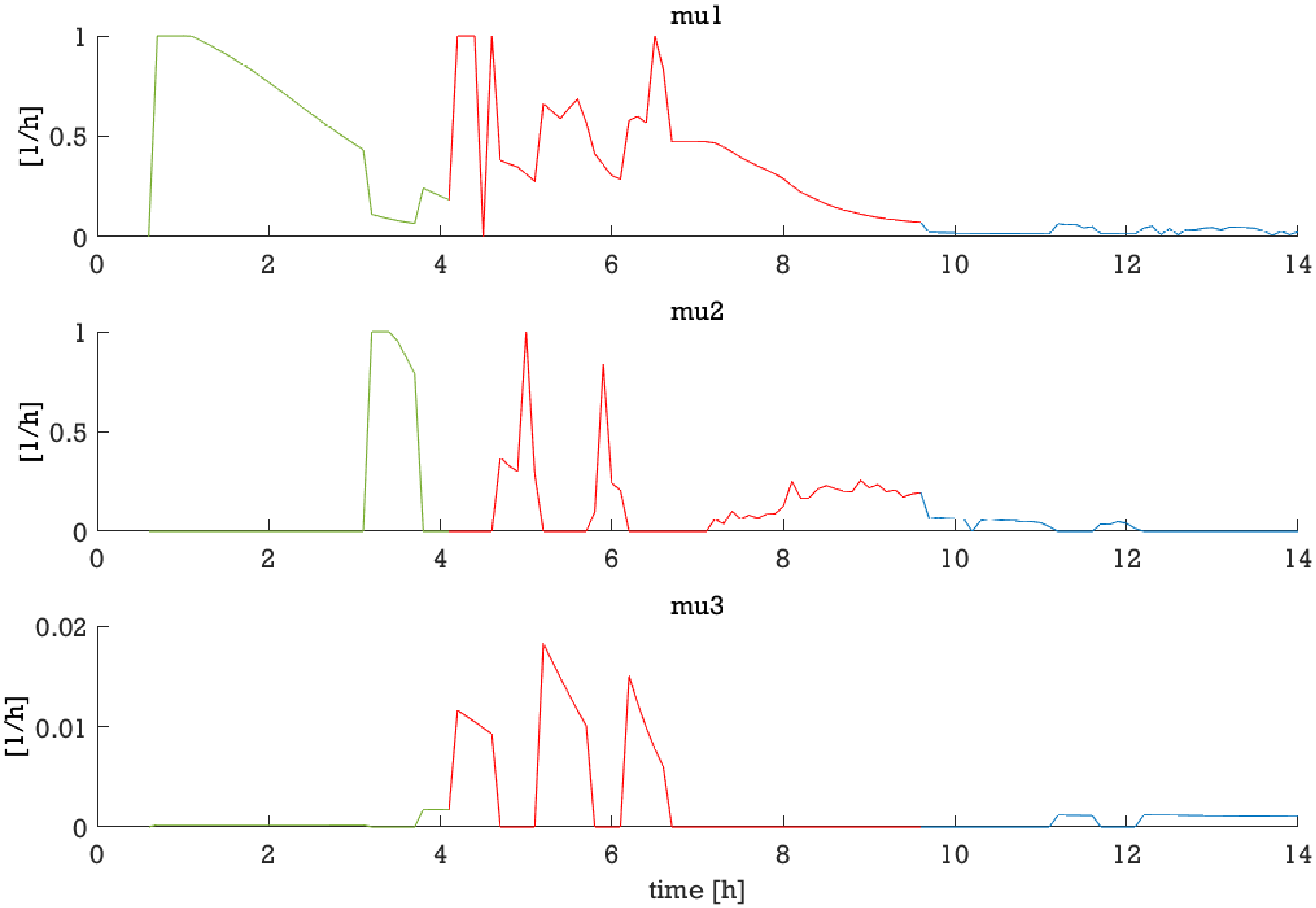


Fig. 6 Simulation results – models values of the three specific growth rates for the three phases: batch phase – green lines, exponential growth phase – red lines, and stationary phase – blue lines.

Biochemical model

$$\frac{dV}{dt} = F_{in}$$

$$\frac{d(S.V)}{dt} = -q_S(X.V) + F_{in}S_f$$

$$\frac{d(X.V)}{dt} = \mu(X.V)$$

$$\frac{d(A.V)}{dt} = (q_a^p - q_a^c)(X.V)$$

$$\frac{d(C_O V)}{dt} = -q_O(X.V) + K_{La}(N).V.(C_O^* - C_O)$$

$$q_S = q_S^{\max}\frac{S}{k_S + S}$$

(1)

When $q_S < q_S^{crit}$

$$\mu = q_S Y_{SX}^{oxid}$$

$$q_a^p = 0$$

$$q_a^c = \min\left(\frac{q_a^{c.\max}A}{k_a + A}, \frac{q_O^{\max} - q_S Y_{OG}}{Y_{OA}}\right)$$

$$q_O = q_S Y_{OG}$$

When $q_S \geq q_S^{crit}$

$$\mu = q_S^{crit} Y_{SX}^{oxid} + (q_S - q_S^{crit}) Y_{SX}^{ferm}$$

$$q_a^p = (q_S - q_S^{crit}) Y_{SA}$$

$$q_a^c = 0$$

$$q_O = q_S^{crit} Y_{OG}$$

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

(2)

$$\frac{dV}{dt} = F_{in};$$

$$\frac{d(S.V)}{dt} = -\varphi_1 + F_{in}S_f;$$

$$\frac{d(X.V)}{dt} = k_1\varphi_1;$$

$$\frac{d(C_o V)}{dt} = -k_3\varphi_1 + Q_{in}.V;$$

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 $q_s < q_{s,crit}$

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

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

(3)

$$\frac{dV}{dt} = F_{in};$$

$$\frac{d(S.V)}{dt} = -\varphi_1 - \varphi_2 + F_{in}S_f;$$

$$\frac{d(X.V)}{dt} = k_1\varphi_1 + k_2\varphi_2;$$

$$\frac{d(C_o V)}{dt} = -k_3\varphi_1 + Q_{in}.V;$$

$$\frac{d(OUR.V)}{dt} = k_4\varphi_1 + k_5\varphi_2.$$

where

$$k_1 = Y_{SX}^{oxid},$$

$$k_2 = Y_{SX}^{fe}$$

$$k_3 = Y_{OG};$$

$$k_4 = k_1 k_3 q_S^{crit}$$

$$k_5 = k_2 k_3 q_S^{crit}$$

$$\varphi_1 = q_S^{crit} V.X;$$

$$\varphi_2 = (q_S - q_S^{crit}) V.X;$$

$$OUR.V = k_3 q_S^{crit} X.V;$$

$$Q_{in}.V = K_{La}(N)(C_O^* - C_O).V;$$

Operational model when $q_s >= q_{s,crit}$

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

$$\frac{dZ_1}{dt} = -\frac{1}{k_3} Q_{in}.V + F_{in} S_f \qquad Z_1 = -\frac{1}{k_3} C_o.V + S.V$$

$$\frac{dZ_2}{dt} = \frac{k_1}{k_3} Q_{in}.V \qquad Z_2 = \frac{k_1}{k_3} C_O.V + X.V$$

Adaptive Biomass Observer for the case $q_S < q_S^{crit}$

$$\frac{dV}{dt} = F_{in}$$

$$\frac{dZ_1}{dt} = -\frac{1}{k_3} Q_{in}.V + F_{in} S_f \qquad (4)$$

$$\frac{dZ_2}{dt} = \frac{k_1}{k_3} Q_{in}.V$$

$$\hat{X} = (Z_2 + k_1 Z_1)/V$$

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

$$Z_1 = -\frac{1}{k_3} C_o V - \frac{1}{k_3} OUR.V + S.V$$

$$Z_2 = \frac{k_1 q_S^{crit} - k_1}{k_2 k_3 q_S^{crit}} C_o V + \frac{k_1 q_S^{crit} - k_2}{k_2 k_3 q_S^{crit}} OUR.V + XV$$

$$\frac{dZ_1}{dt} = -\frac{1}{k_3} Q_{in}.V + F_{in} S_f$$

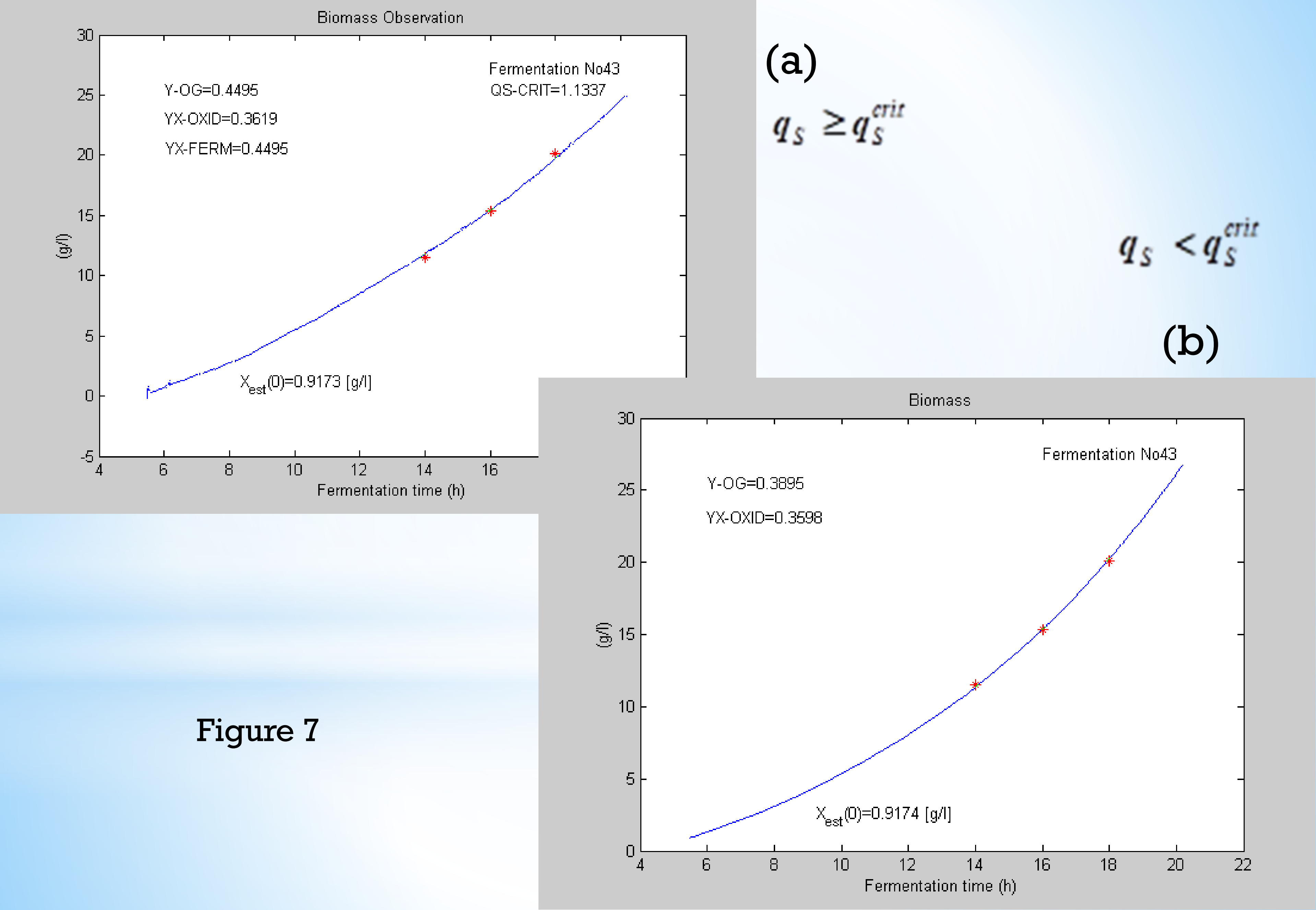
$$\frac{dZ_2}{dt} = \frac{k_1 q_S^{crit} - k_1}{k_2 .k_3 q_S^{crit}} Q_{in}.V$$

Adaptive Biomass Observer for the case $q_S \geq q_S^{crit}$

$$\begin{aligned} \frac{dV}{dt} &= F_{in} \\ \frac{dZ_1}{dt} &= -\frac{1}{k_3} Q_{in}.V + F_{in} S_f \\ \frac{dZ_2}{dt} &= \frac{k_1 q_S^{crit} - k_1}{k_2 k_3 q_S^{crit}} Q_{in}.V \end{aligned} \tag{5}$$

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

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

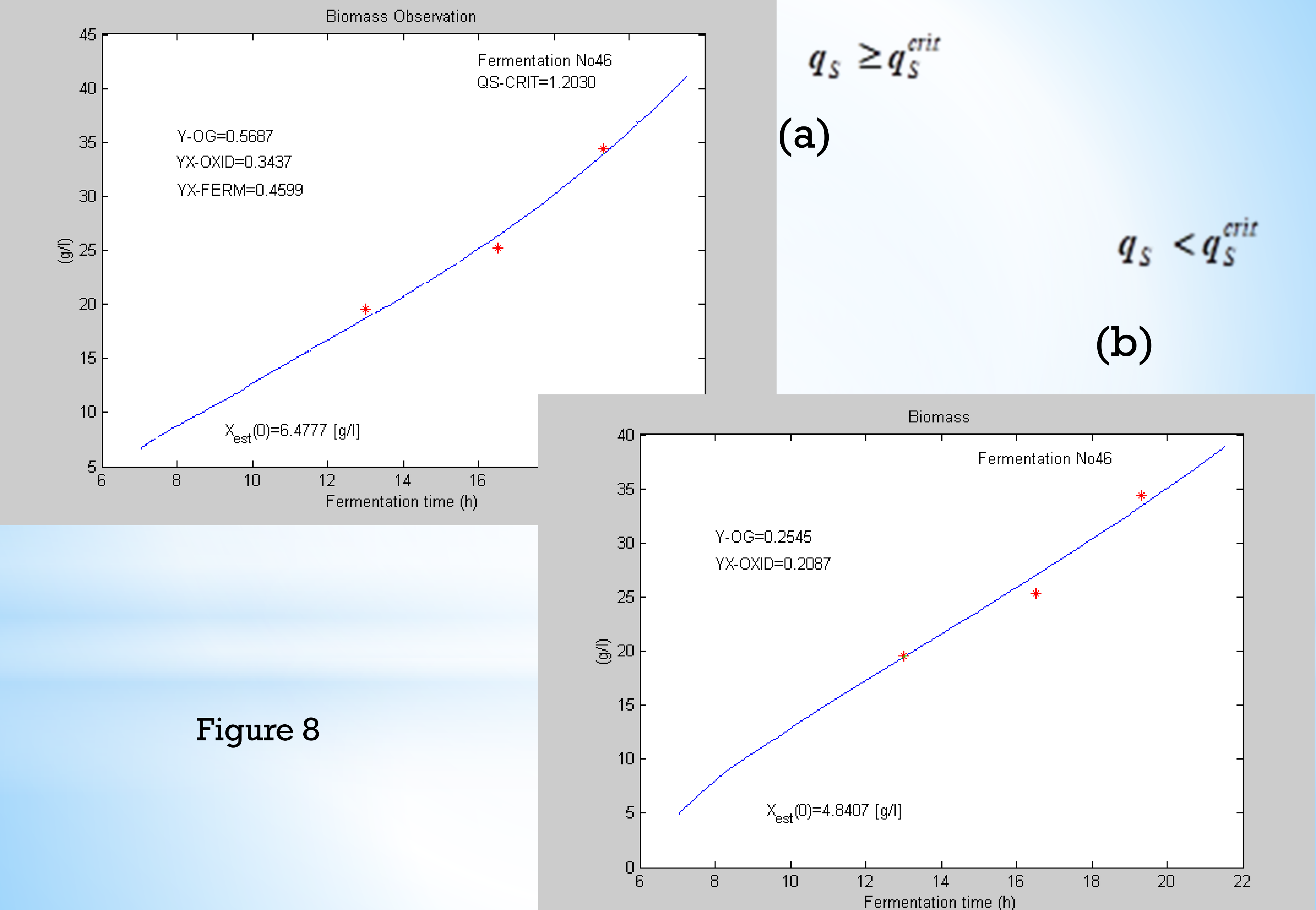


$q_S \geq q_S^{crit}$

$q_S < q_S^{crit}$

Figure 7

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



$q_S \geq q_S^{crit}$

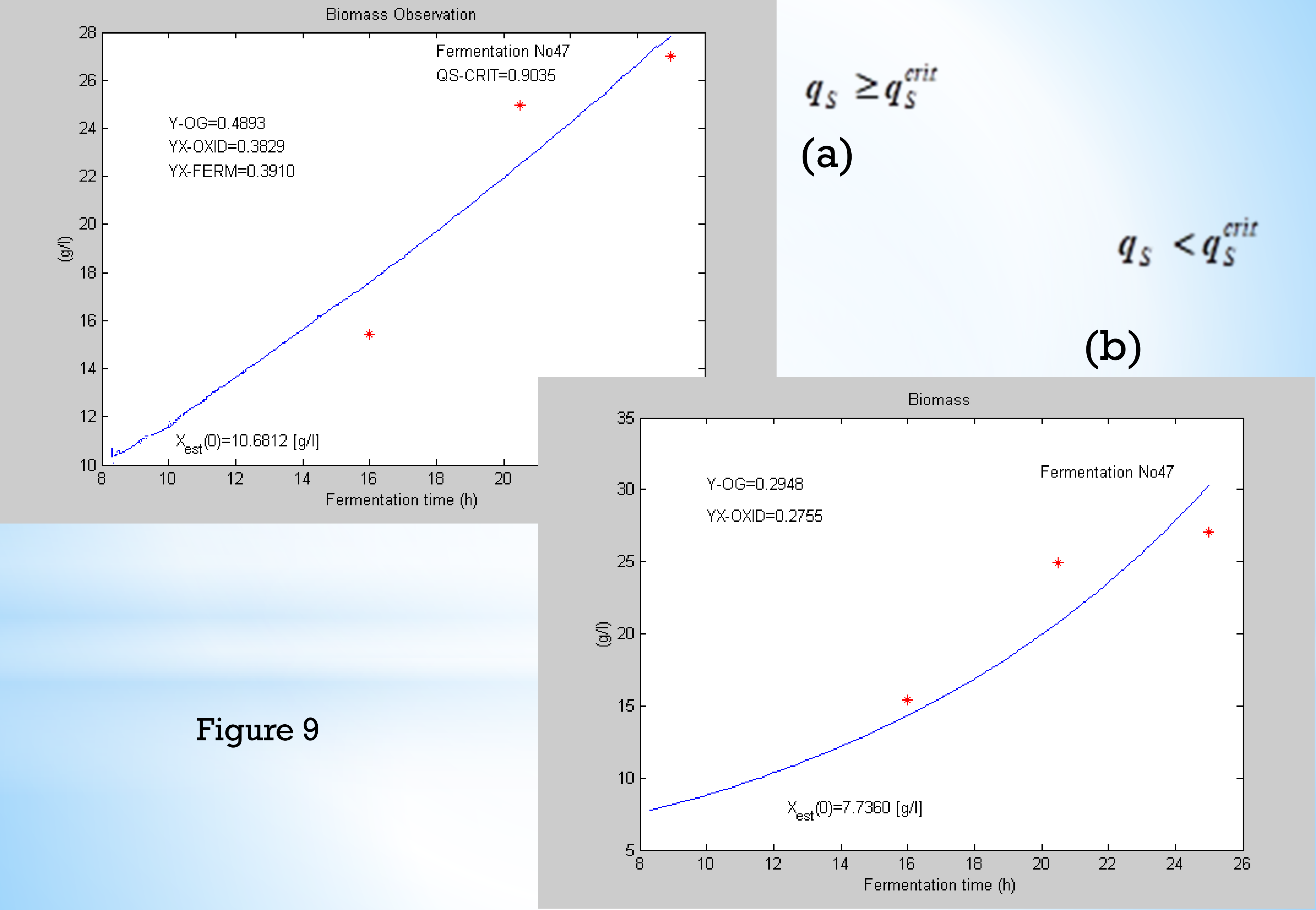
(a)

$q_S < q_S^{crit}$

(b)

Figure 8

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



$q_S \geq q_S^{crit}$

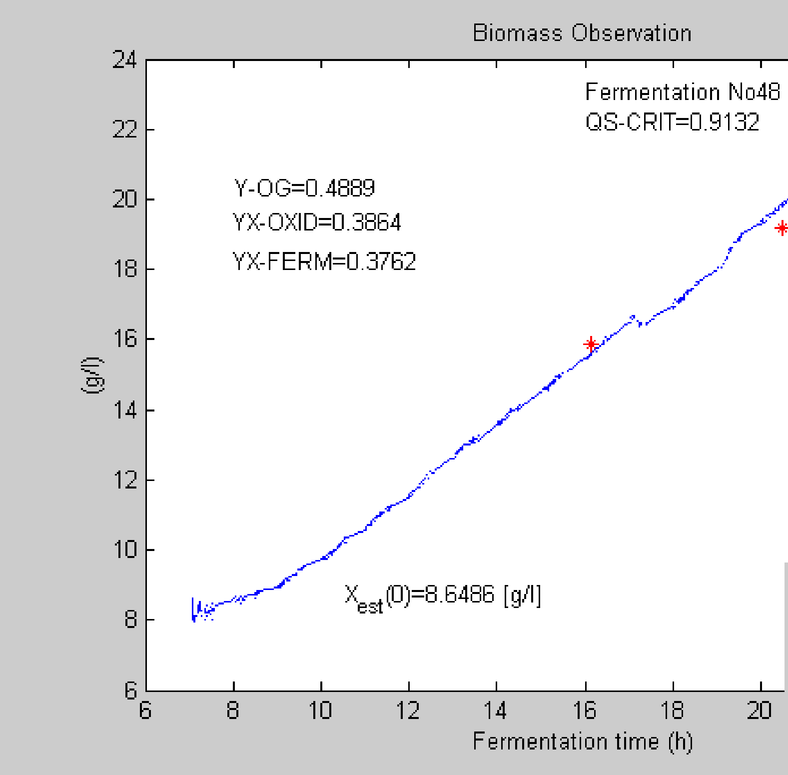
(a)

$q_S < q_S^{crit}$

(b)

Figure 9

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



$q_S \geq q_S^{crit}$

(a)

$q_S < q_S^{crit}$

(b)

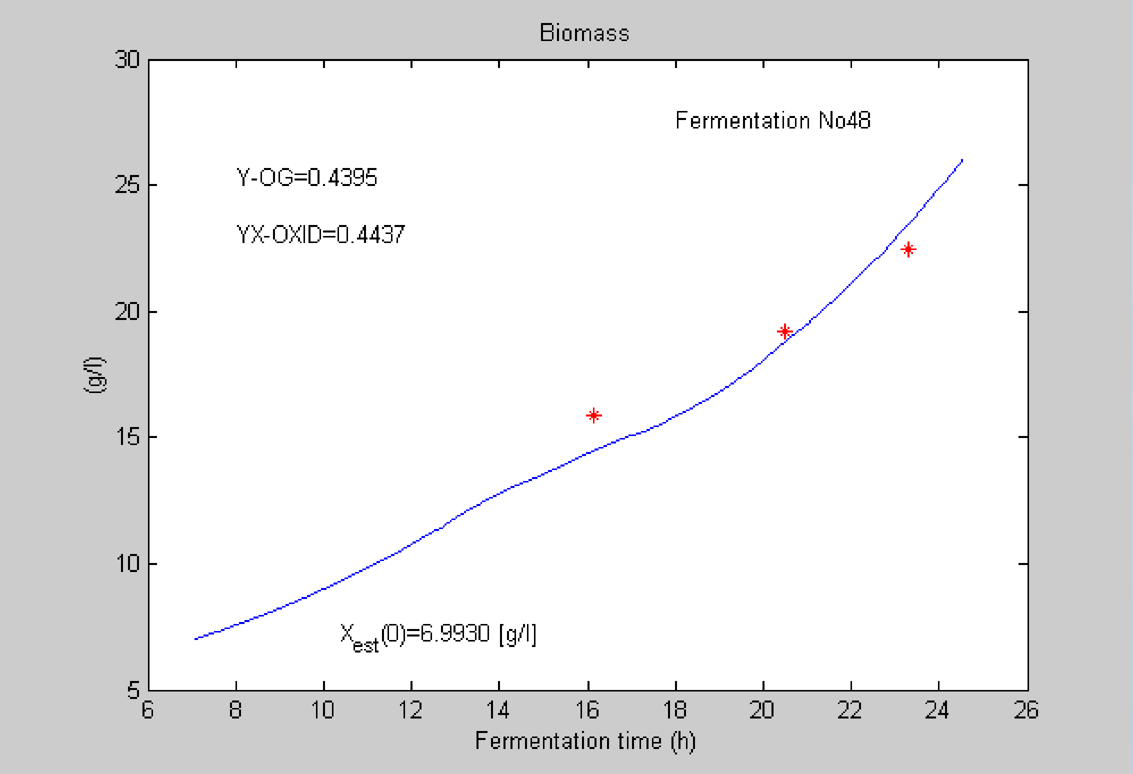


Figure 10

Adaptive Control of Protein Production Bioprocess with Three Physiological States

Operational process model describing three physiological states

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

(6)

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

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

Ra = 0 oxidative growth on glucose

Ra > 0 oxidative-fermentative growth on glucose

Ra < 0 oxidative growth on acetate

Adaptive Control of Protein Production Bioprocess with Three Physiological States

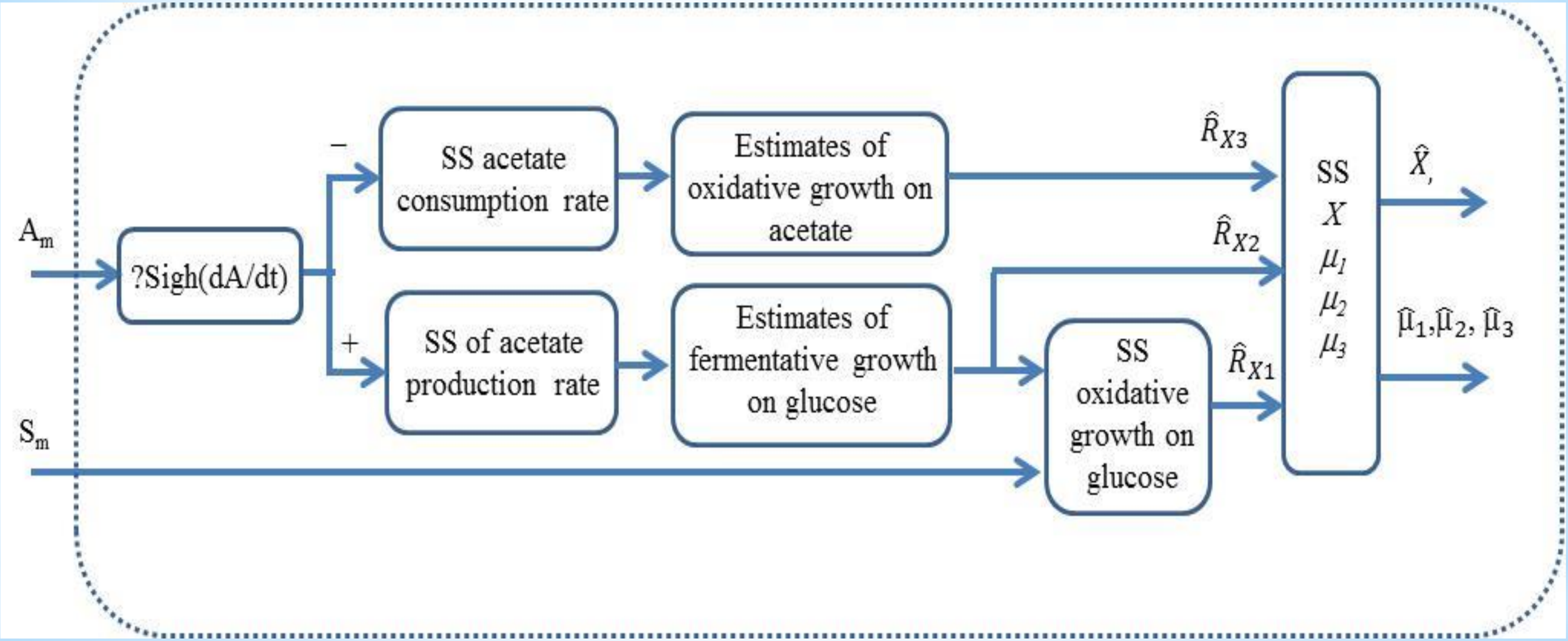


Figure 11 Cascade structure of the software sensor for monitoring of three metabolic states

Adaptive Control of Protein Production Bioprocess with Three Physiological States

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

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

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

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

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

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

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

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

Figure 12 Observer based estimators grouped, depending on the physiological state of the process

Adaptive Control and Results

$$\boldsymbol{F} = \frac{\boldsymbol{W}.\left(-\boldsymbol{\lambda}(\boldsymbol{S}^{*} - \boldsymbol{S}_{\boldsymbol{m}}) + \boldsymbol{k}_1\widehat{\boldsymbol{R}}_{X1} + \boldsymbol{k}_2\widehat{\boldsymbol{R}}_{X2} + \boldsymbol{k}_4\widehat{\boldsymbol{R}}_{X3}\right)}{\boldsymbol{S}_{\boldsymbol{in}} - \boldsymbol{S}_{\boldsymbol{m}}} \qquad (7)$$

Adaptive Control of Protein Production Bioprocess with Three Physiological States

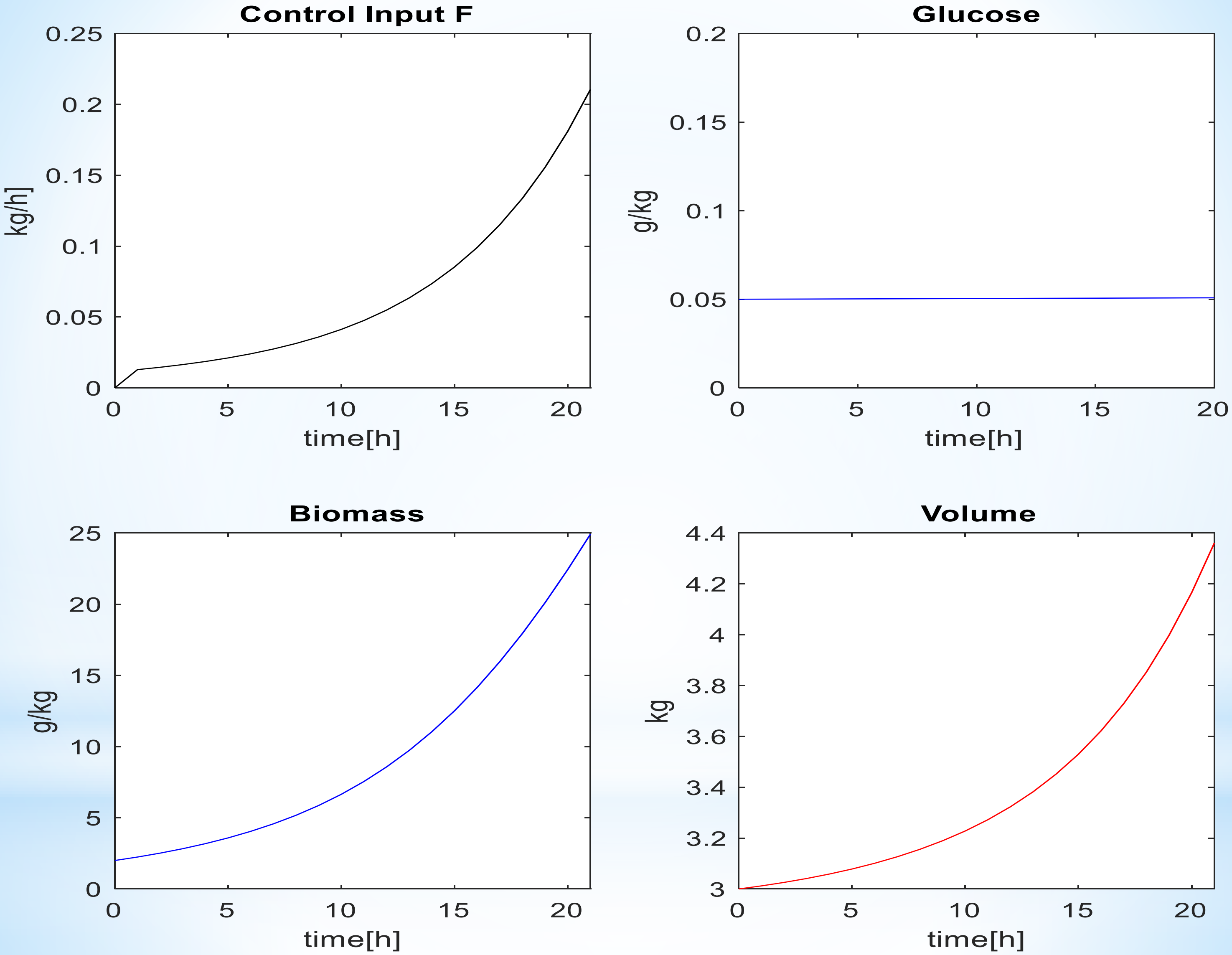


Figure 13 Linearizing control algorithm investigation

Adaptive Control of Protein Production Bioprocess with Three Physiological States

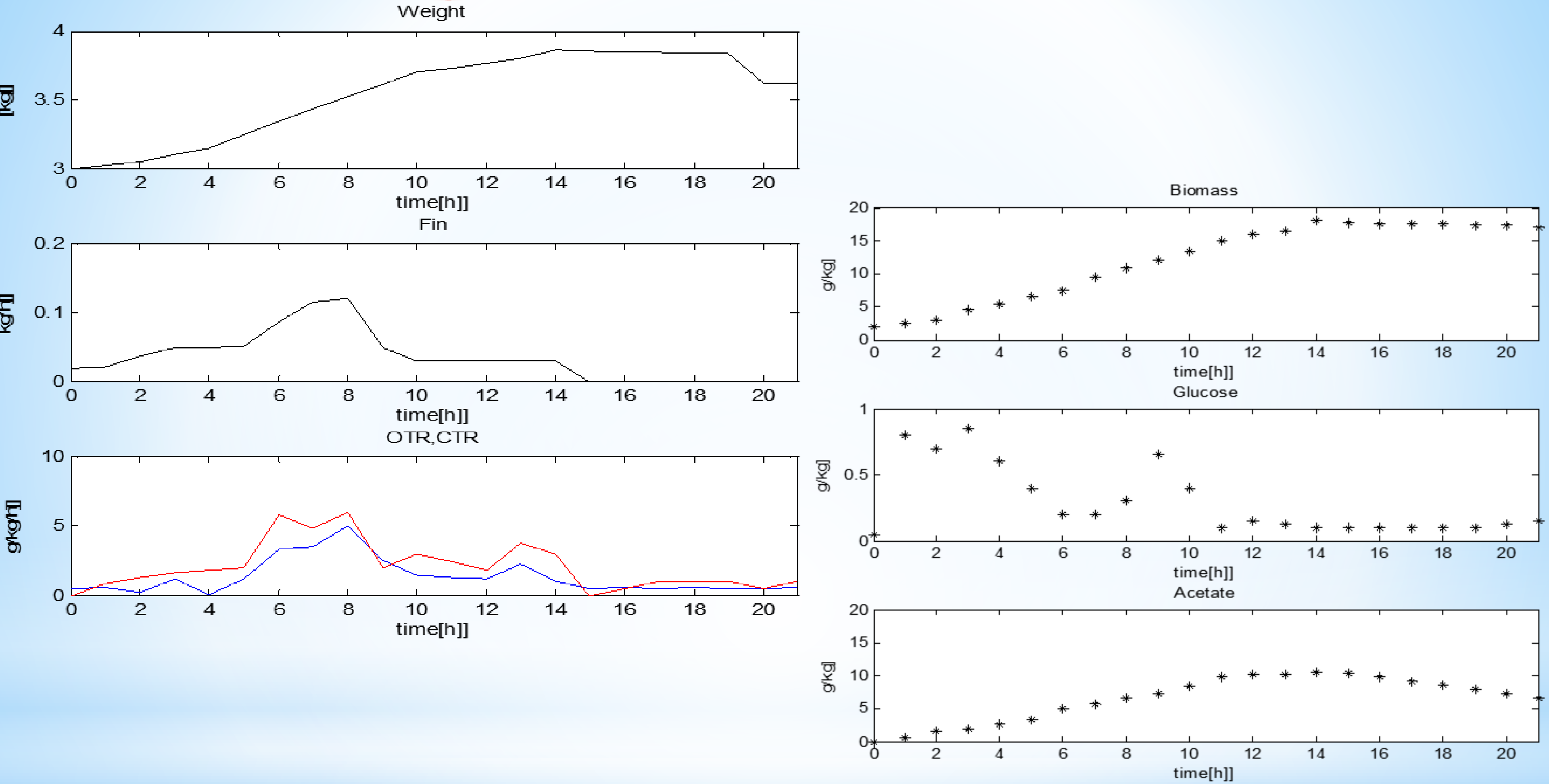


Figure 14 Open loop control for the fermentation according the experimental data

Further research and work on the system

Two modules have been planned initially as part of the interactive system InSEMCoBio.
The module for model parameter identification of the system is currently under development. It will be further expanded with new hybrid metaheuristic algorithms and different models of cultivation processes.

The work on the system InSEMCoBio will continue further by developing the second module for an adaptive control design

Acknowledgements

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