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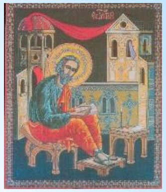
**Почетен член на "Съвета на Европейската научна и културна общност”**



# Interactive System for Education in Modelling and Control of Bioprocesses

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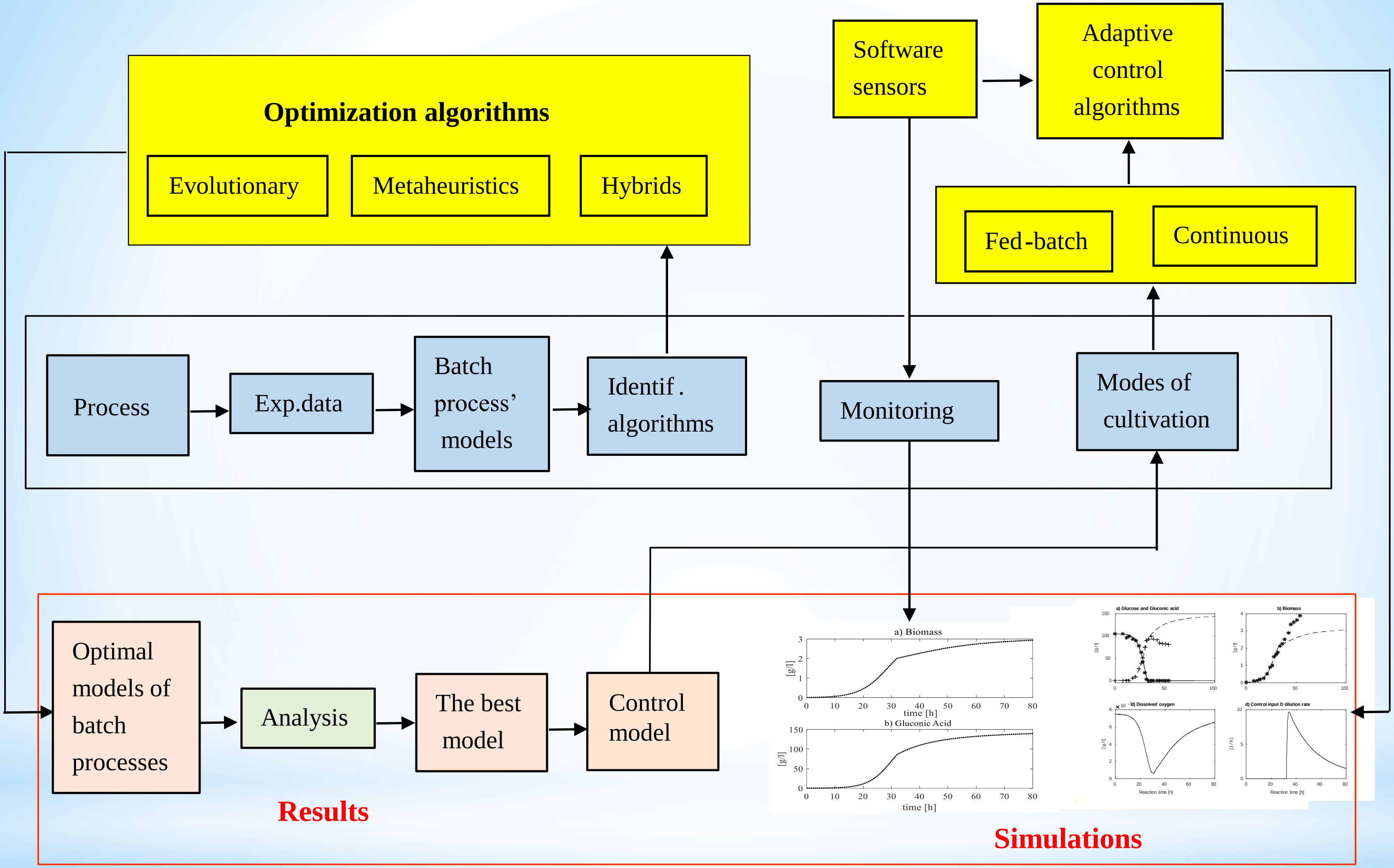
Sibiu, Roumania



**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

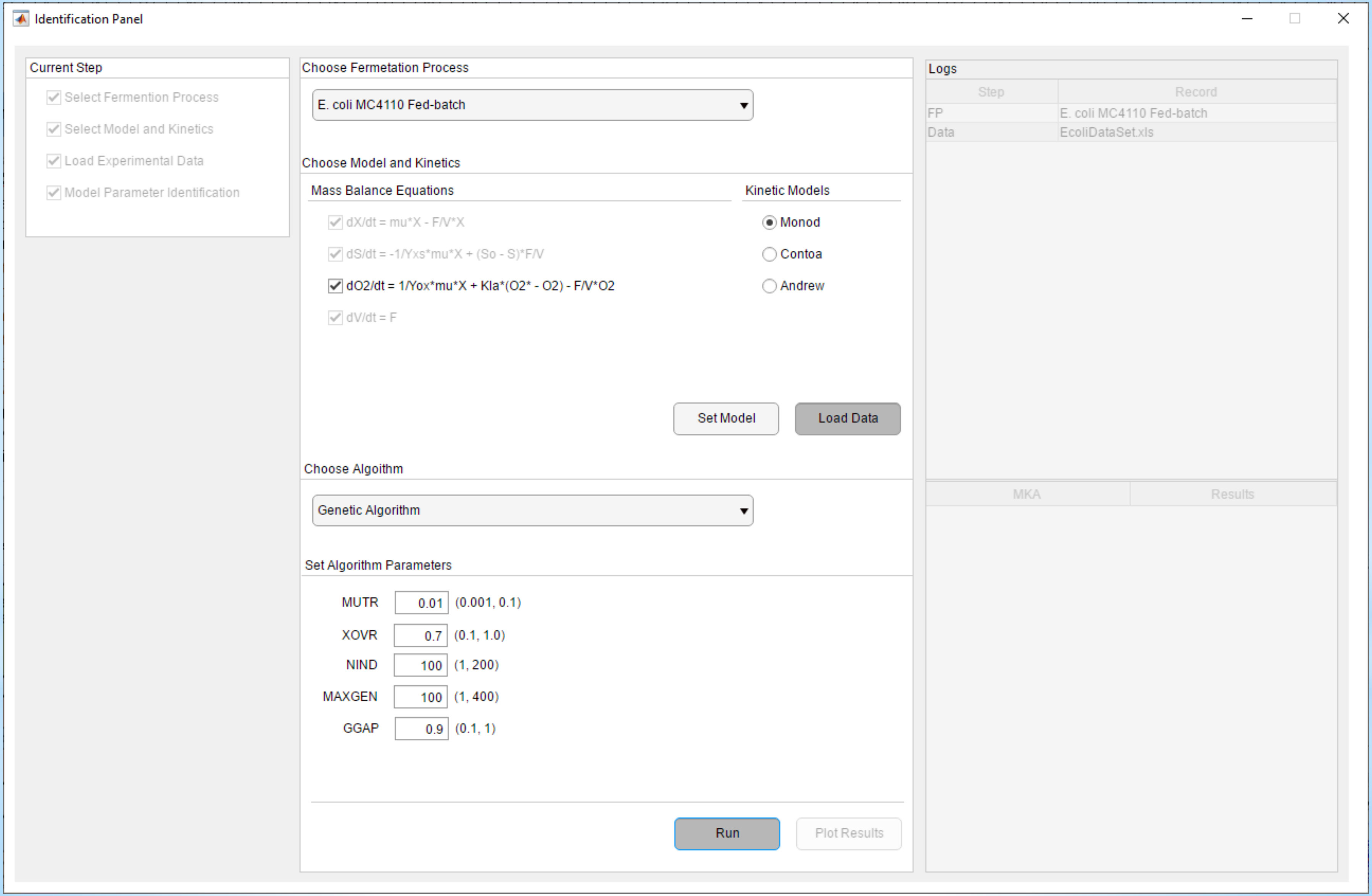
Control model

**Results**

**Simulations**

Fig. 1. Open source system InSEMCoBio

# Setting up a fermentation process model and metaheuristic algorithm parameters in InSEMCoBio



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

Choose Algoithm

Genetic Algorithm ▾

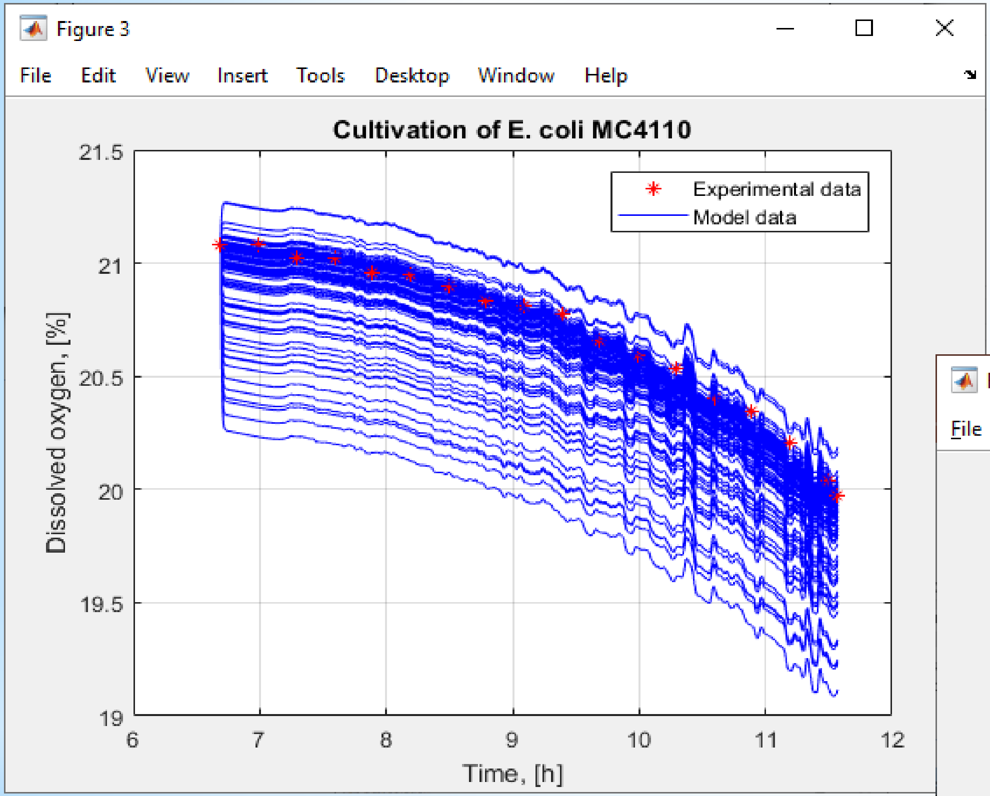
Set Algorithm Parameters

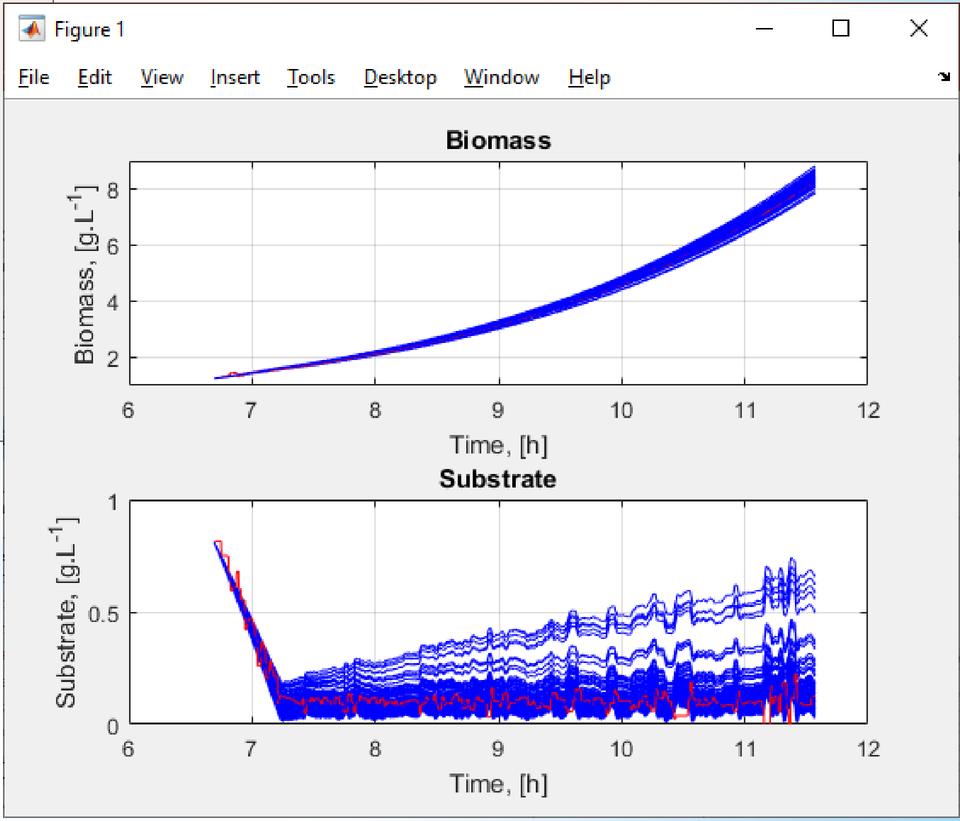
MUTR 0.01 (0.001, 0.1)
XOVR 0.7 (0.1, 1.0)
NIND 100 (1, 200)
MAXGEN 100 (1, 400)
GGAP 0.9 (0.1, 1)

Logs

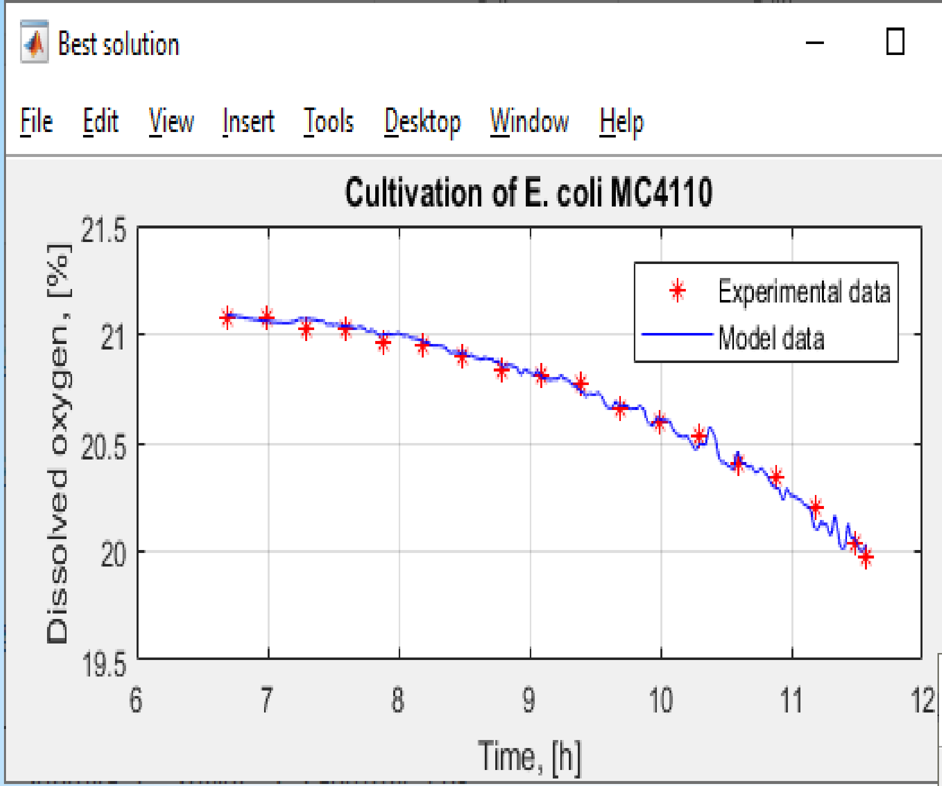
| Step | Record |
|---|---|
| FP | E. coli MC4110 Fed-batch |
| Data | EcoliDataSet.xls |

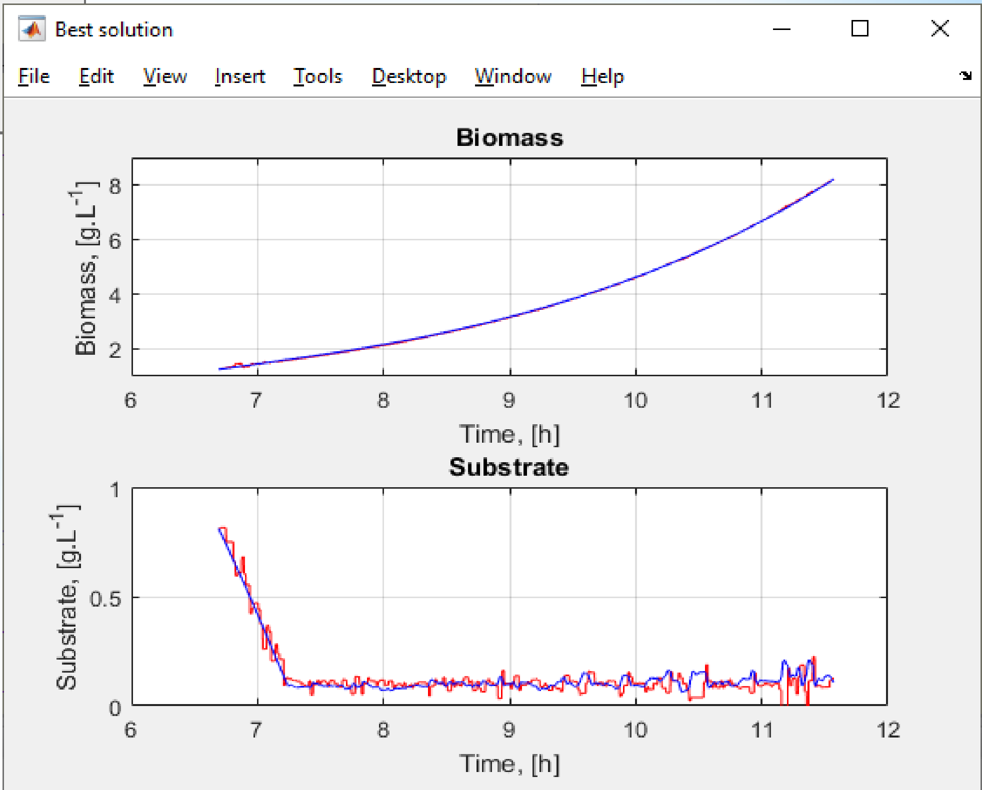
# Visualizing the results of the identification procedure in InSEMCoBio





# Visualizing the results of the identification procedure in InSEMCoBio





# *Model Identification and Monitoring of E. Coli* fed-batch cultivation for extracellular production of bacterial phytase

# Oxidative-fermentative growth model on glucose

$$k_1 S + k_5 O \overset{\mu 1}{\rightarrow} X + k_8 C$$

**Oxidative growth on glucose**

**Model**

$$R_{ac} = 0$$

$$\frac{d}{dt}\begin{bmatrix} X \\ S \\ A \\ O \\ C_r \end{bmatrix} = \begin{bmatrix} 1 \\ -k_1 \\ 0 \\ -k_5 \\ k_8 \end{bmatrix} \mu_1 X - D \begin{bmatrix} X \\ S \\ A \\ O \\ C_r \end{bmatrix} + \frac{F_{in,S}}{W}\begin{bmatrix} 0 \\ S_{in} \\ 0 \\ 0 \\ 0 \end{bmatrix} + \begin{bmatrix} 0 \\ 0 \\ 0 \\ OTR \\ -CTR \end{bmatrix}$$

$$q_s = q_{s,\max} S / (K_S + S)$$

$$\mu_1 = q_s / k_1$$

**Marker**

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

$$k_1 S + k_5 O \overset{\mu 1}{\rightarrow} X + k_8 C$$

$$k_2 S + k_6 O \overset{\mu 2}{\rightarrow} X + k_3 C + k_9 A$$

**Oxidative-fermentative growth on glucose**

**Model**

$$R_{ac} > 0$$

$$\frac{d}{dt}\begin{bmatrix} X \\ S \\ A \\ O \\ C_r \end{bmatrix} = \begin{bmatrix} 1 & 1 \\ -k_1 & -k_2 \\ 0 & k_3 \\ -k_5 & -k_6 \\ k_8 & k_9 \end{bmatrix} \begin{bmatrix} \mu_1 \\ \mu_2 \end{bmatrix} X - D \begin{bmatrix} X \\ S \\ A \\ O \\ C_r \end{bmatrix} + \frac{F_{in,S}}{W}\begin{bmatrix} 0 \\ S_{in} \\ 0 \\ 0 \\ 0 \end{bmatrix} + \begin{bmatrix} 0 \\ 0 \\ 0 \\ OTR \\ -CTR \end{bmatrix}$$

$$\mu_1 = q_{s,crit} / k_1 \qquad \mu_2 = (q_s - q_{s,crit}) / k_2 \qquad q_{s,crit} = \frac{q_{o,\max}}{k_{os}} \frac{K_{i,o}}{K_{i,o} + A}$$

# Oxidative-fermentative growth model on glucose and oxidative on acetate

$$k_1 S + k_5 O \xrightarrow{\mu 1} X + k_8 C$$

$$k_2 S + k_6 O \xrightarrow{\mu 2} X + k_3 C + k_9 A$$

**Oxidative-fermentative growth on glucose**

**Model**

$$\frac{d}{dt}\begin{bmatrix} X \\ S \\ A \\ O \\ C_r \end{bmatrix} = \begin{bmatrix} 1 & 1 \\ -k_1 & -k_2 \\ 0 & k_3 \\ -k_5 & -k_6 \\ k_8 & k_9 \end{bmatrix} \begin{bmatrix} \mu_1 \\ \mu_2 \end{bmatrix} X - D \begin{bmatrix} X \\ S \\ A \\ O \\ C_r \end{bmatrix} + \frac{F_{in,S}}{W} \begin{bmatrix} 0 \\ S_{in} \\ 0 \\ 0 \\ 0 \end{bmatrix} + \begin{bmatrix} 0 \\ 0 \\ 0 \\ OTR \\ -CTR \end{bmatrix}$$

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

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

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

$$R_{ac} > 0$$

**Marker**

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

$$k_1 S + k_5 O \xrightarrow{\mu 1} X + k_8 C$$

$$k_4 A + k_7 O \xrightarrow{\mu_3} X + k_{10} C$$

**Oxidative growth on glucose and acetate**

$$R_{ac} < 0$$

$$\frac{d}{dt}\begin{bmatrix} X \\ S \\ A \\ O \\ C_r \end{bmatrix} = \begin{bmatrix} 1 & 1 \\ -k_1 & 0 \\ 0 & -k_4 \\ -k_5 & -k_7 \\ k_8 & k_{10} \end{bmatrix} \begin{bmatrix} \mu_1 \\ \mu_3 \end{bmatrix} X - D \begin{bmatrix} X \\ S \\ A \\ O \\ C_r \end{bmatrix} + \frac{F_{in,S}}{W} \begin{bmatrix} 0 \\ S_{in} \\ 0 \\ 0 \\ 0 \end{bmatrix} + \begin{bmatrix} 0 \\ 0 \\ 0 \\ OTR \\ -CTR \end{bmatrix}$$

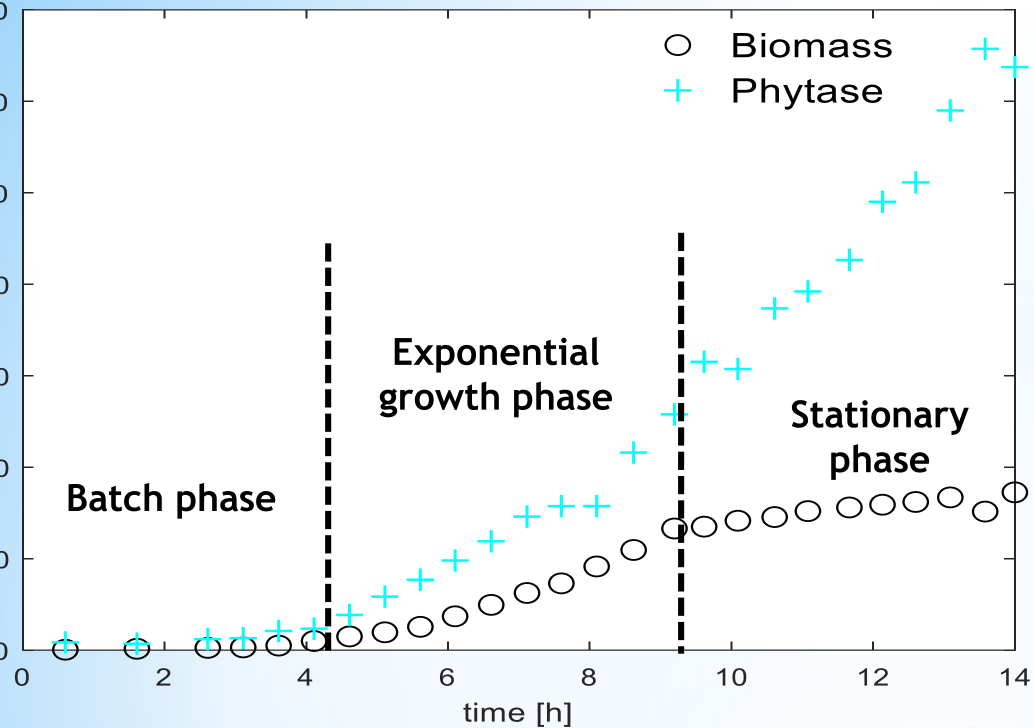
**Model**

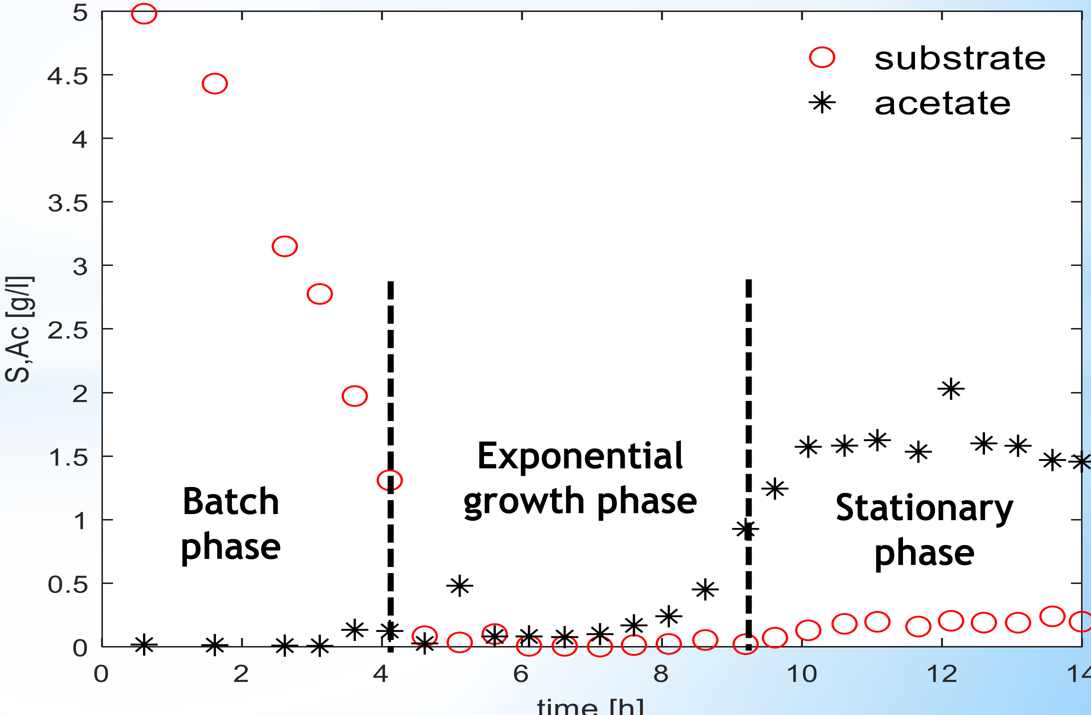
$$\mu_1 = q_{s,crit} / k_1 \qquad \mu_3 = q_{ac} / k_4$$

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

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

# Experimental data

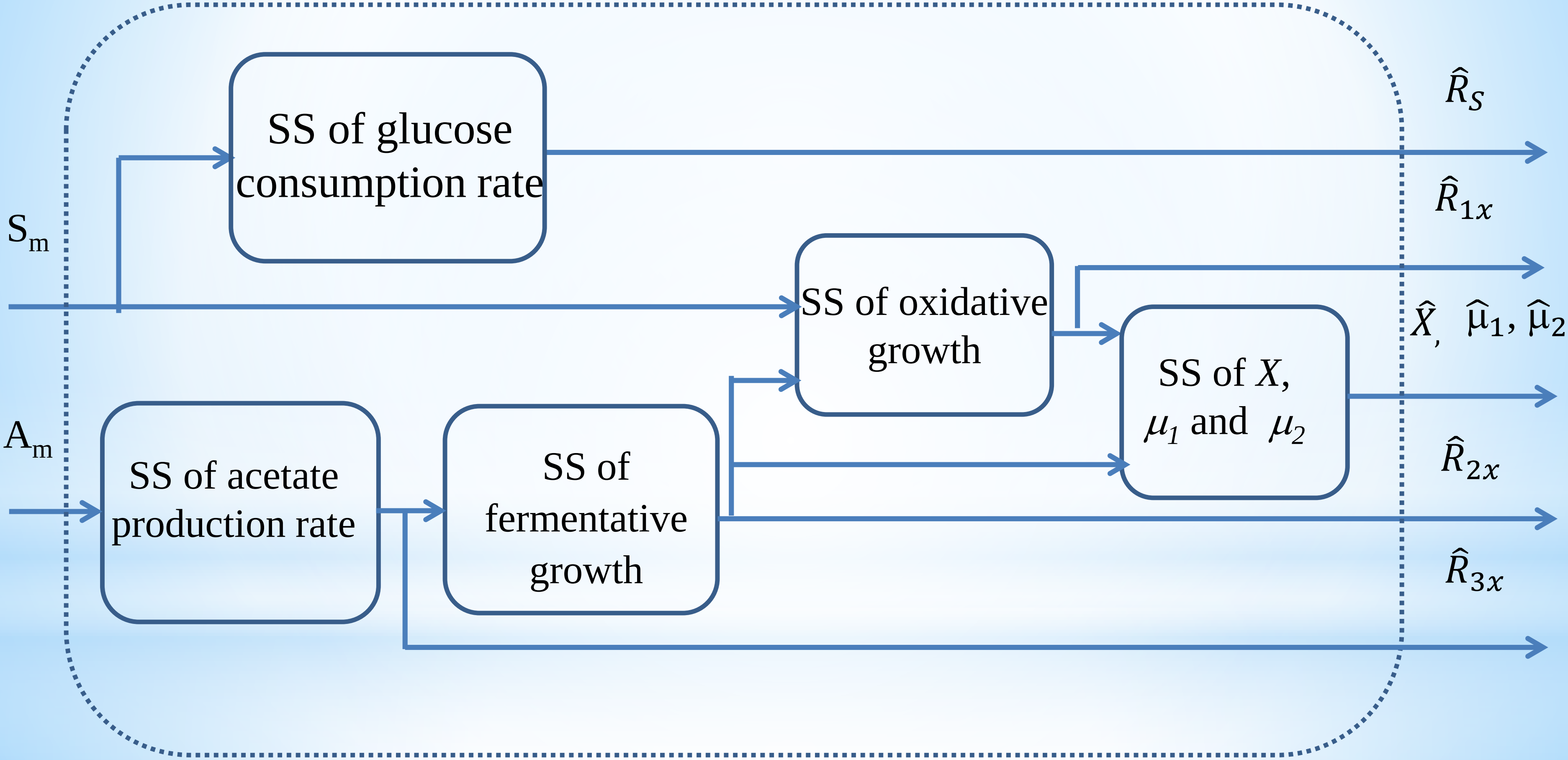




# Comparison of the coefficients of the best models for the three phases

| | $q_{smax}$ | $k_s$ | $k_{is}$ | $q_{omax}$ | $k_{os}$ | $k_{io}$ | $q_{acmax}$ | $k_a$ | $k_{ia}$ | $k_1$ | $k_2$ | $k_3$ | $k_4$ | $k_5$ | $k_6$ | $k_7$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **1 phase** | 4.19 | 0.19 | 5.54 | 1.1 | 2.15 | 0.088 | 0.082 | 1.17 | - | 3.69 | 0.557 | 0.187 | 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 | 0.049 | 4.1 | 2.88 | 1.52 | 0.5 |
| **3 phase** | 77.11 | 0.47 | 12.3 | 2.1 | 3.29 | 0.134 | 0.0021 | 0.295 | 0.228 | 16.6 | 11.66 | 0.42 | 9.9 | 39.45 | 9.53 | 0.56 |

# Scheme of the cascade software sensor of the process kinetics



$S_m$

$A_m$

SS of glucose consumption rate

SS of oxidative growth

SS of acetate production rate

SS of fermentative growth

SS of $X$, $\mu_1$ and $\mu_2$

$\hat{R}_S$

$\hat{R}_{1x}$

$\hat{X}$, $\hat{\mu}_1$, $\hat{\mu}_2$

$\hat{R}_{2x}$

$\hat{R}_{3x}$

# On-line estimation of acetate production and consumption rates

## Acetate production

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

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

## Acetate consumption

$$\frac{d\hat{A}}{dt} = -\hat{R}_{ac} - DA_m + \lambda_1(A_m - \hat{A})$$

$$\frac{d\hat{R}_{ac}}{dt} = -\lambda_2(A_m - \hat{A})$$

# On-line estimation of $R_{1x}$ , $R_{2x,}$ $R_{3x}$ and X

$$\frac{d\hat{S}}{dt} = -k_1\hat{R}_{1X} - k_2\hat{R}_{ap}/k_3 - \frac{F}{V}S_m + \frac{F_{in,S}}{V}S_{in} + \lambda_1(S_m - \hat{S})$$

$$\frac{d\hat{R}_{1x}}{dt} = \lambda_2(S_m - \hat{S})$$

$$\hat{R}_{2X} = \hat{R}_{ap}/k_3$$

$$\hat{\mu}_1 = \hat{R}_{1X}/\hat{X}$$

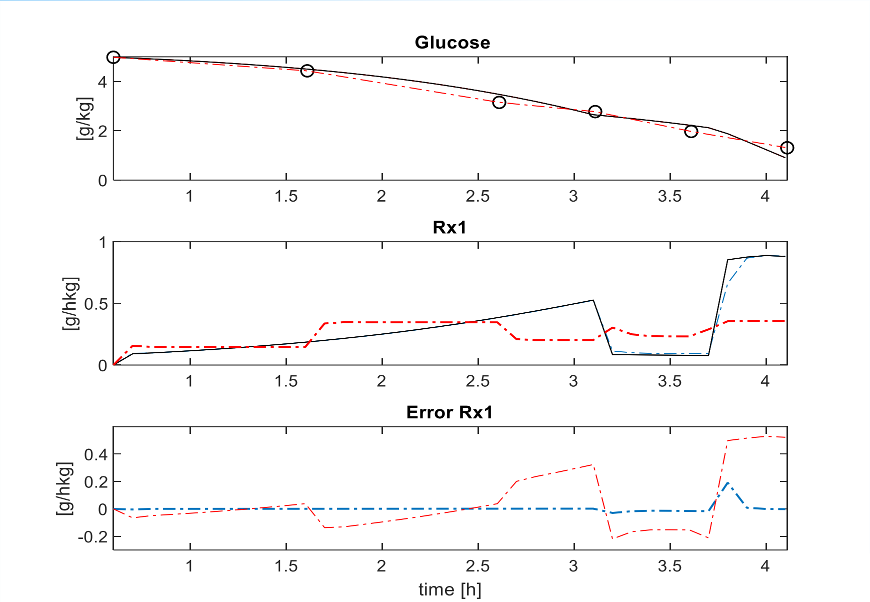
$$\hat{R}_{3X} = -\hat{R}_{ac}/k_4$$

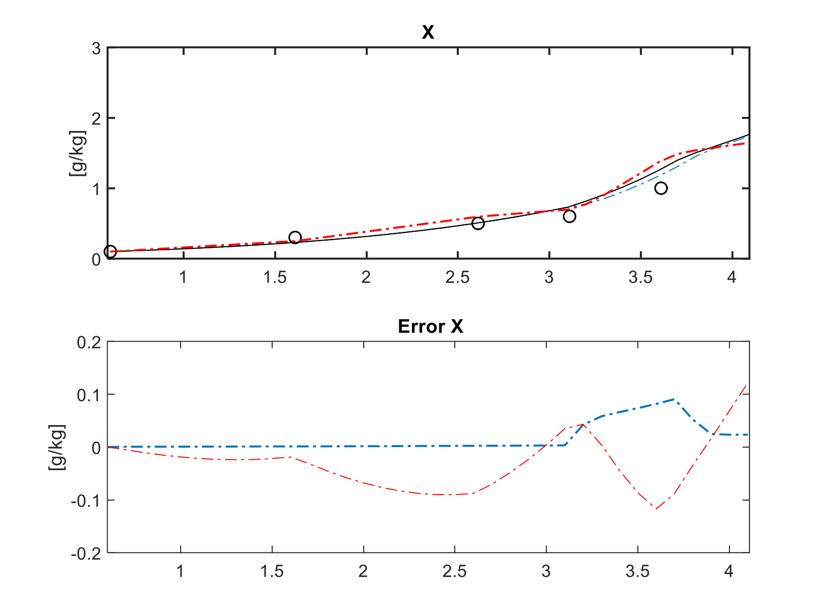
$$\hat{\mu}_2 = \hat{R}_{2X}/\hat{X}$$

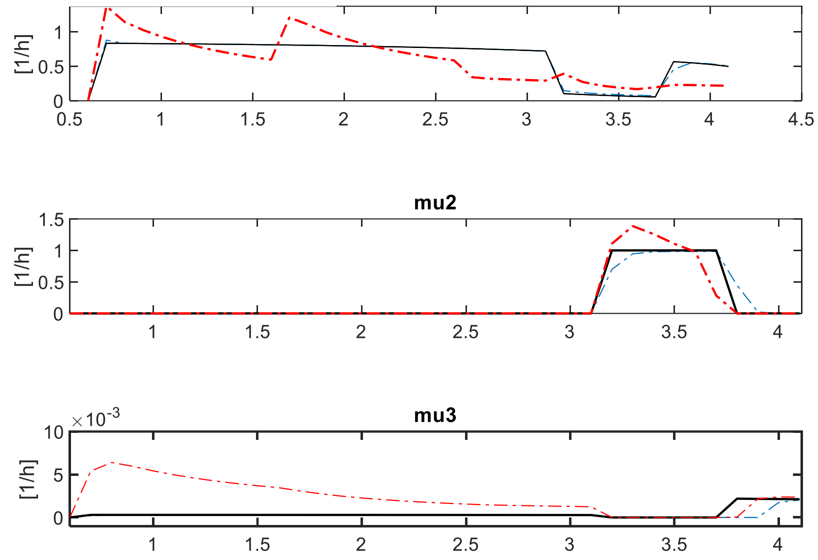
$$\frac{d\hat{X}}{dt} = \hat{R}_{1X} + \hat{R}_{2X} + \hat{R}_{3X} - D\hat{X}$$

$$\hat{\mu}_3 = \hat{R}_{3X}/\hat{X}$$

# Monitoring results– I phase





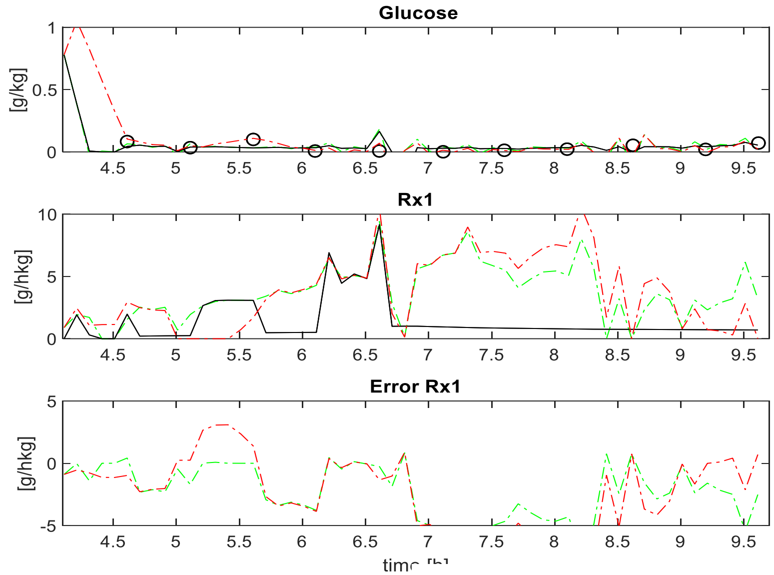


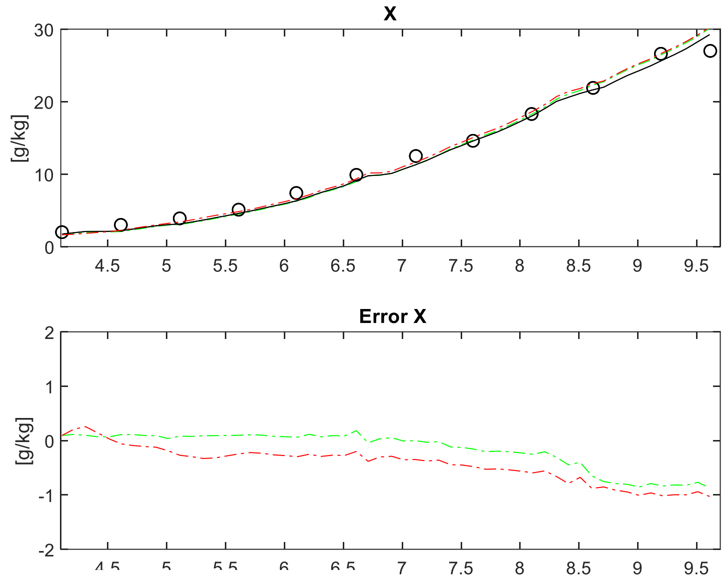
Tuning parameters
C1=50;
C2=50;

Black lines- model data

SS results: with red lines - inputs are experimental data, with blue lines – inputs are model data

# Monitoring results– II phase



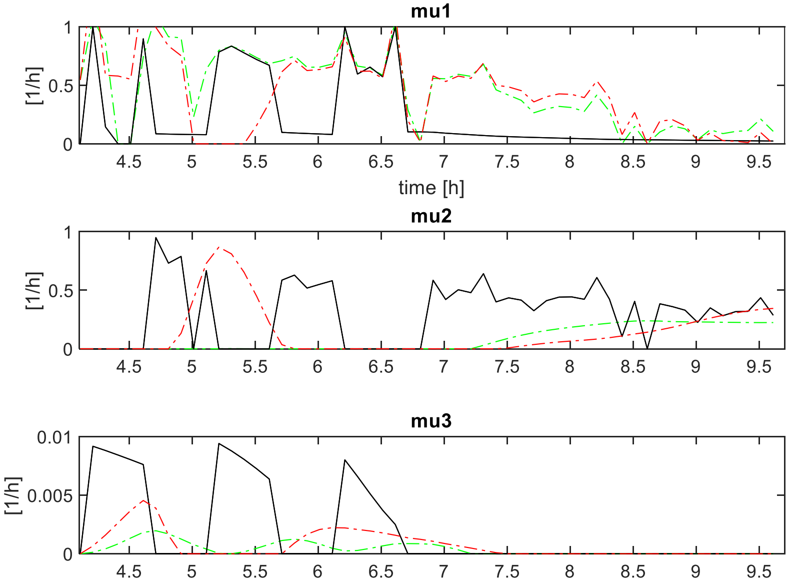


SS tuning parameters

C1= 54.7764;

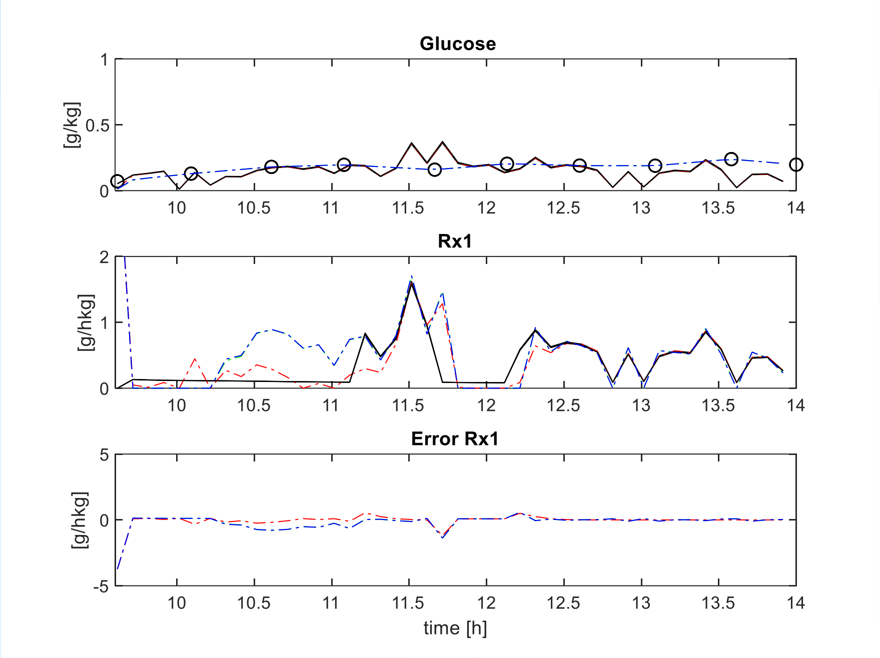
C2= 2.7926;

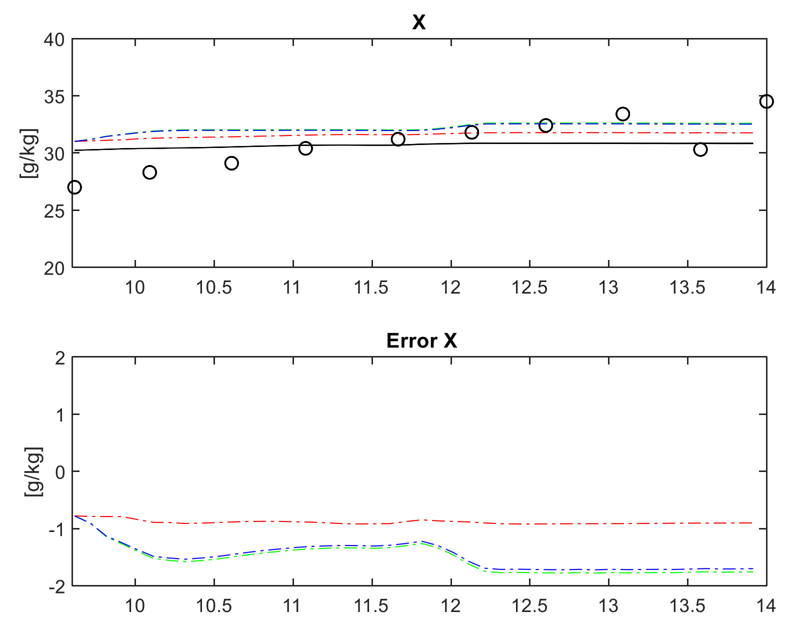
Black lines- model data



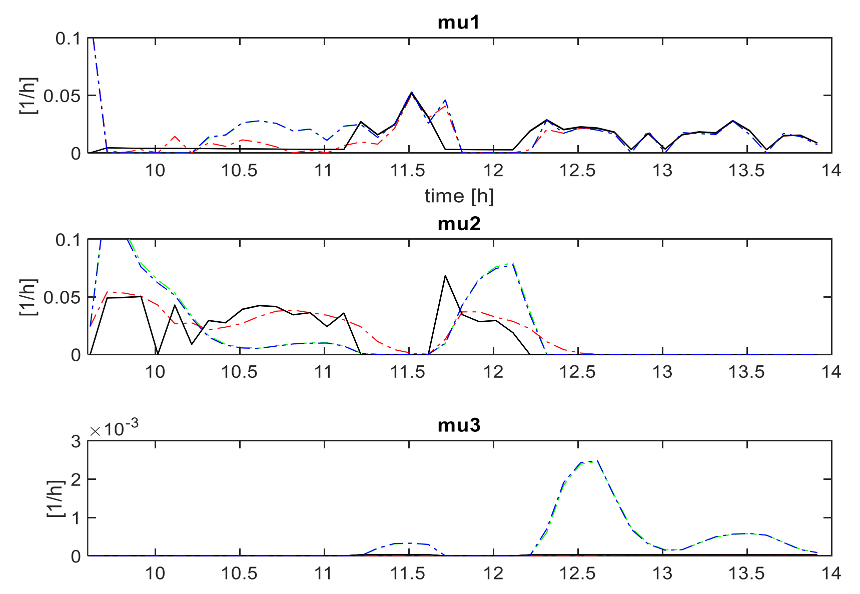
SS results: with red lines - inputs are experimental data, with green lines – inputs are model data

# Monitoring results– III phase





SS tuning parameters
C1=77.0215
C2=25
red lines – model data
green lines (C2=1.3) и blue lines (C2=25)
– experimental data



# Comparison of the coefficients of the best models for the three phases

| | $q_{smax}$ | $k_s$ | $k_{is}$ | $q_{omax}$ | $k_{os}$ | $k_{io}$ | $q_{acmax}$ | $k_a$ | $k_{ia}$ | $k_1$ | $k_2$ | $k_3$ | $k_4$ | $k_5$ | $k_6$ | $k_7$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **1 phase** | 4.19 | 0.19 | 5.54 | 1.1 | 2.15 | 0.088 | 0.082 | 1.17 | - | 3.69 | 0.557 | 0.187 | 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 | 0.049 | 4.1 | 2.88 | 1.52 | 0.5 |
| **3 phase** | 77.11 | 0.47 | 12.3 | 2.1 | 3.29 | 0.134 | 0.0021 | 0.295 | 0.228 | 16.6 | 11.66 | 0.42 | 9.9 | 39.45 | 9.53 | 0.56 |

# A Methodology for Simultaneously State and Kinetics Observation of a Class Controllable Bioproceses

Oxidative growth on main carbon source, with specific growth rate $\mu_1$:

$$S \xrightarrow{\mu 1} X$$

Fermentative growth on main carbon source, with specific growth rate $\mu_2$:

$$S \xrightarrow{\mu 2} X + P_{int}$$

Oxidative growth on intermediate product, with specific growth rate $\mu_3$:

$$P_{int} \xrightarrow{\mu 3} X$$

As can be seen from the last reaction, the main source of carbon is depleted and biomass increases at the expense of the intermediate. Its concentration is usually low, leading to insignificant growth of biomass and low productivity of the target product. To avoid this reaction, the processes are controlled by feeding on a basic carbon source.

This is done through two modes of control – fed-batch or continuous modes of cultivations.

For the considered class processes is accepted that transport dynamics, main carbon source and intermediate product are on-line measured.

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# A Methodology for Simultaneously State and Kinetics Observation of a Class Bioproceses

From what has been said so far, it is clear that the considered processes have two specific growth rates and total biomass.

The new idea is to present the biomass as consisting of two parts - the first one is connected with oxidative growth on main carbon source, $X_1$, the second – with fermentative growth on main carbon source, $X_2$. The full biomass concentration will be the sum of $X_1$ and $X_2$:

$X = X_1 + X_2$

The reaction scheme for the considered case is rewritten:

<u>Oxidative growth on main carbon source, with specific growth rate $\mu_1$</u>:

$$S \overset{\mu 1}{\rightarrow} X_1$$

<u>Fermentative growth on main carbon source, with specific growth rate $\mu_2$</u>:

$$S \overset{\mu 2}{\rightarrow} X_2 + P_{int}$$

## *Estimator of oxidative growth of biomass $X_1$ with specific growth $\mu_1$*

$$\frac{dZ_1}{dt} = -\frac{F}{V}Z_1 + \frac{F}{V}S_{in}$$

$$\frac{d\hat{S}}{dt} = (S - Z_1)\hat{\mu}_1 - \frac{F}{V}(S - S_{in}) - \textcolor{red}{k_2}\hat{\mu}_2\hat{X}_2 + w_1(S - \hat{S})$$

$$\frac{d\hat{\mu}_1}{dt} = w_2(S - Z_1)(S - \hat{S})$$

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

## *Estimator of fermentative growth of biomass $X_2$ with specific growth $\mu_2$*

$$\frac{dZ_2}{dt} = -\frac{F}{V}Z_2$$

$$\frac{d\hat{P}_{int}}{dt} = (P_{int} - Z_2)\hat{\mu}_2 - \frac{F}{V}P_{int} + \gamma_1\left(P_{int} - \hat{P}_{int}\right)$$

$$\frac{d\hat{\mu}_2}{dt} = \gamma_2\left(P_{int} - \hat{P}_{int}\right)(P_{int} - Z_2)$$

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

## *Observers of biomasses $X_1$ and $X_2$*

$$\frac{d}{dt}\begin{bmatrix}\hat{X}_1\\ \hat{X}_2\end{bmatrix}=\begin{bmatrix}\hat{\mu}_1 & 0\\ 0 & \hat{\mu}_2\end{bmatrix}\begin{bmatrix}\hat{X}_1\\ \hat{X}_2\end{bmatrix}-\frac{F}{V}\begin{bmatrix}\hat{X}_1\\ \hat{X}_2\end{bmatrix}$$

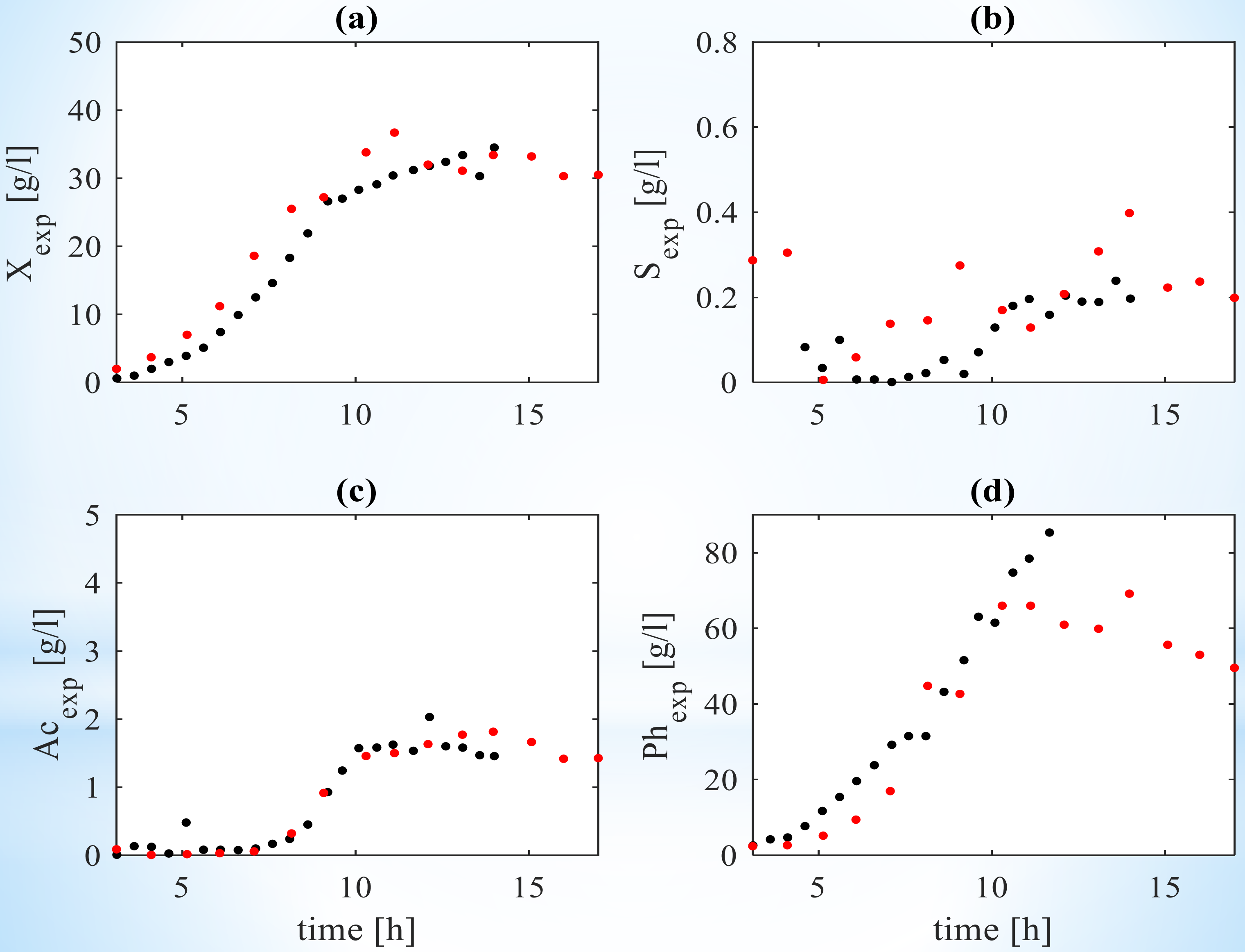
$$\frac{dV}{dt}=F$$

$$\hat{X}=\hat{X}_1+\hat{X}_2$$

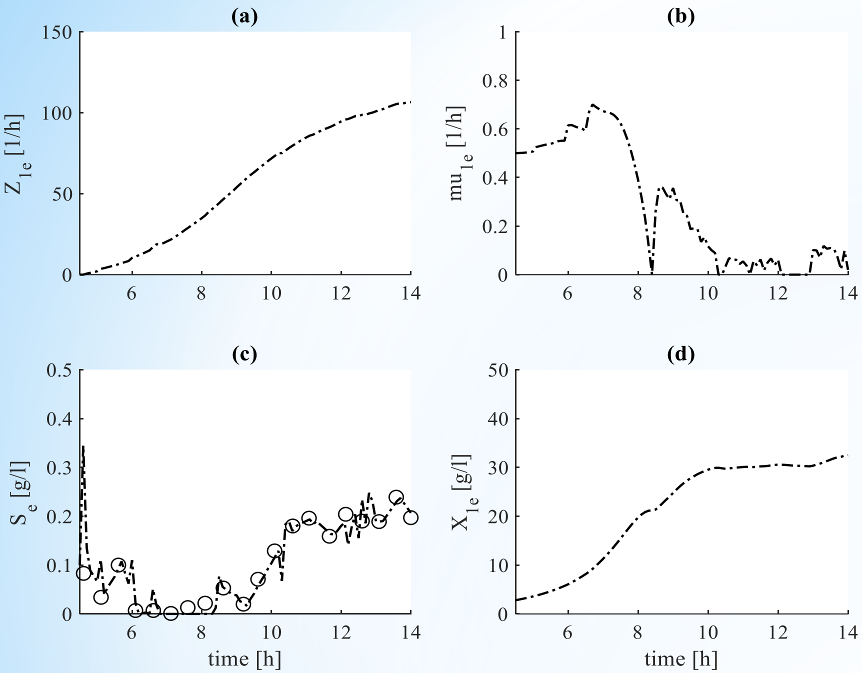
## *Observer of target product*

$$\frac{d\widehat{Ph}}{dt}=k_{ph1}\hat{\mu}_1\hat{X}_1++k_{ph2}\hat{\mu}_2\hat{X}_2-\frac{F}{V}Ph$$

# Two sets of experimental data

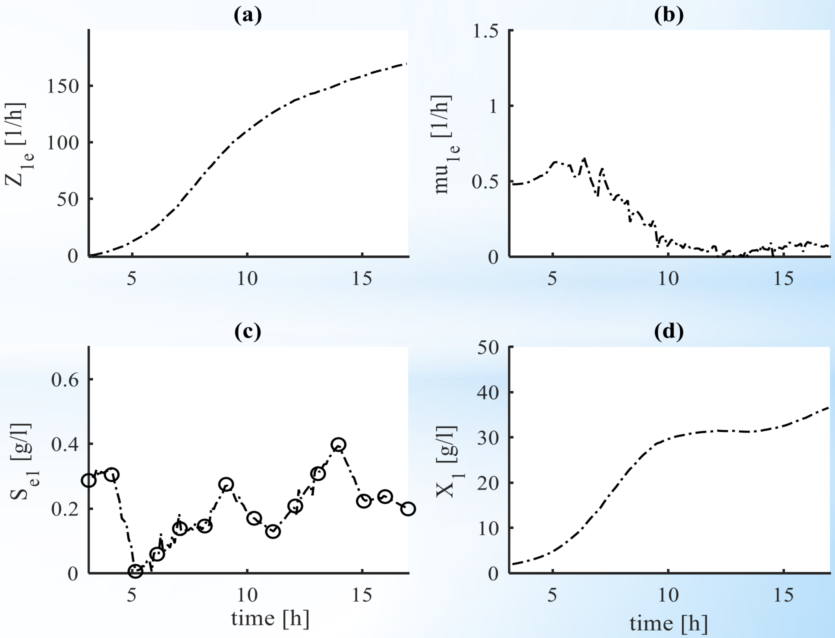


# Estimates of $\mu_1$ and $X_1$

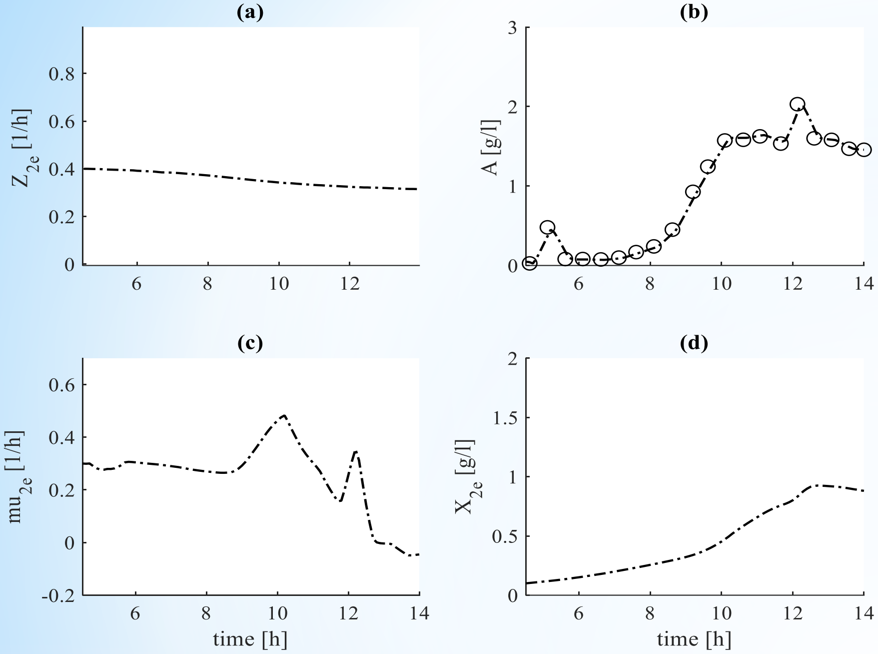


Tuning

Verification

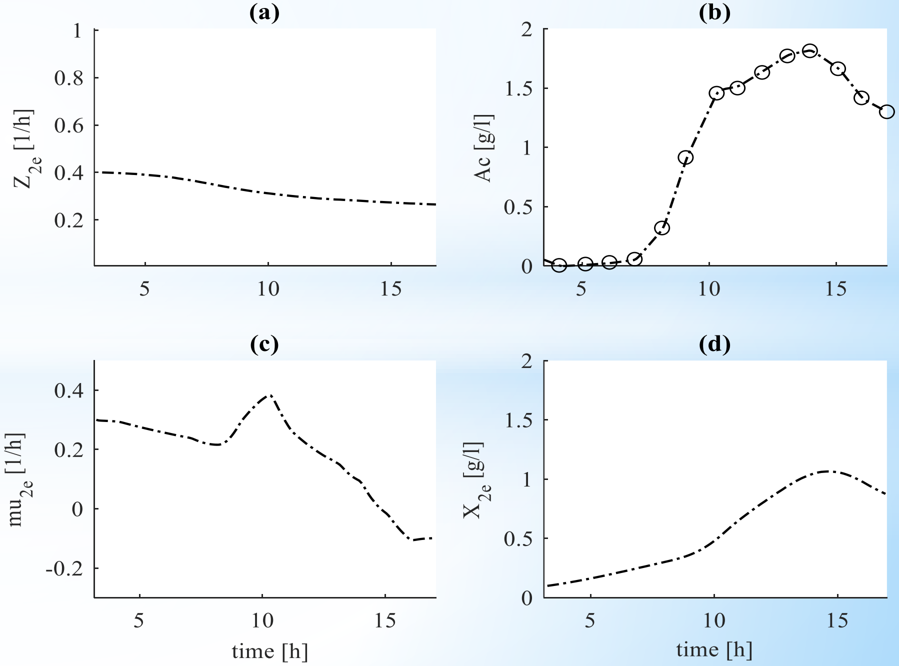


# Estimates of $\mu_2$ and $X_2$

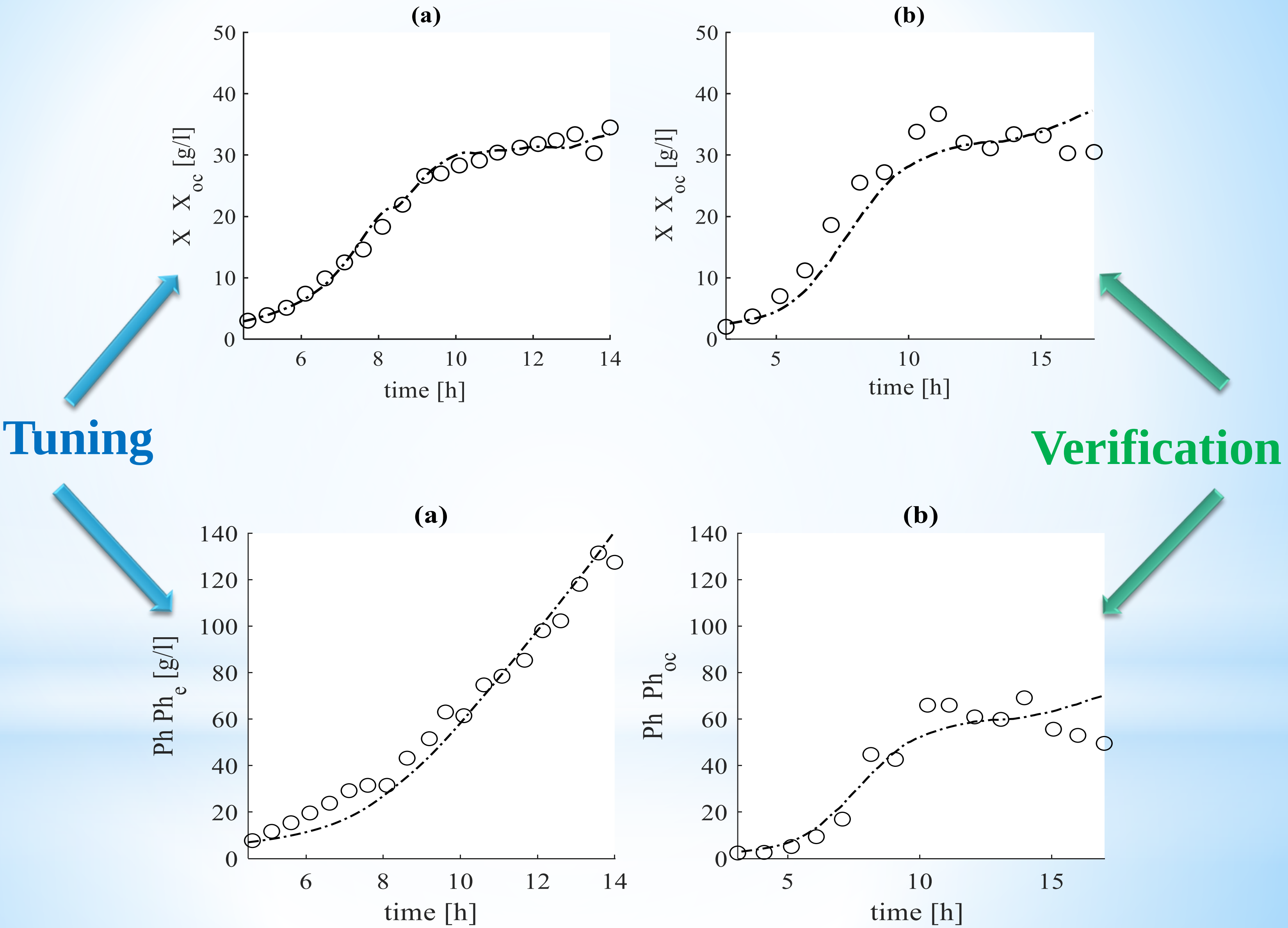


**Tuning**

**Verification**



# Observers of X and Ph



**Tuning**

**Verification**

# *Model Identification*, *Monitoring and Control of Aspergillus Niger* Fermentation for Gluconic Acid Production

# Biochemical model and reaction scheme reduction

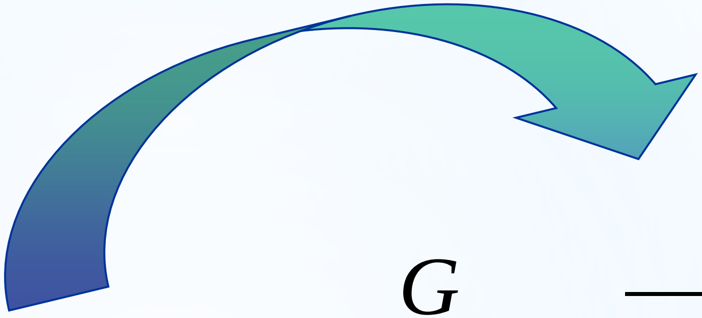
$$G \xrightarrow{R_X} X$$

$$G + O_2 \xrightarrow{R_{GOT}} GOT + H_2O_2$$

$$GOT + H_2O \xrightarrow{R_{GA}} GA$$

$$H_2O_2 \xrightarrow{R_{H_2O_2}^{decom}} H_2O + \frac{1}{2}O_2$$

$$\cancel{GA \xrightarrow{R_{GA}^{decom}} X}$$



$$G \xrightarrow{R_X} X$$

$$G + O_2 \xrightarrow{R_{GA}} GA$$

# Reducted biochemical model

$$\frac{dX}{dt} = R_x;$$

$$\frac{dG}{dt} = -R_x - R_{GOT};$$

$$\frac{dGOT}{dt} = R_{GOT} - R_{GA};$$

$$\frac{dGA}{dt} = R_{GA};$$

$$\frac{dO}{dt} = -R_{GOT} + 0.5R_{H_2O_2} + K_L a(O_2^{\ *} - O_2);$$

$$\frac{dH_2O_2}{dt} = -R_{H_2O_2};$$

where

$$R_{H_2O_2} = R_{GOT} - R_{H_2O_2}^{decom} \ ;$$

$$R_x = \mu_{\max} X \frac{k - X}{k} \ ;$$

$$R_{GA} = \mu_{GA} GA \frac{(k_{GA} - GA)}{k_{GA}} \ ;$$

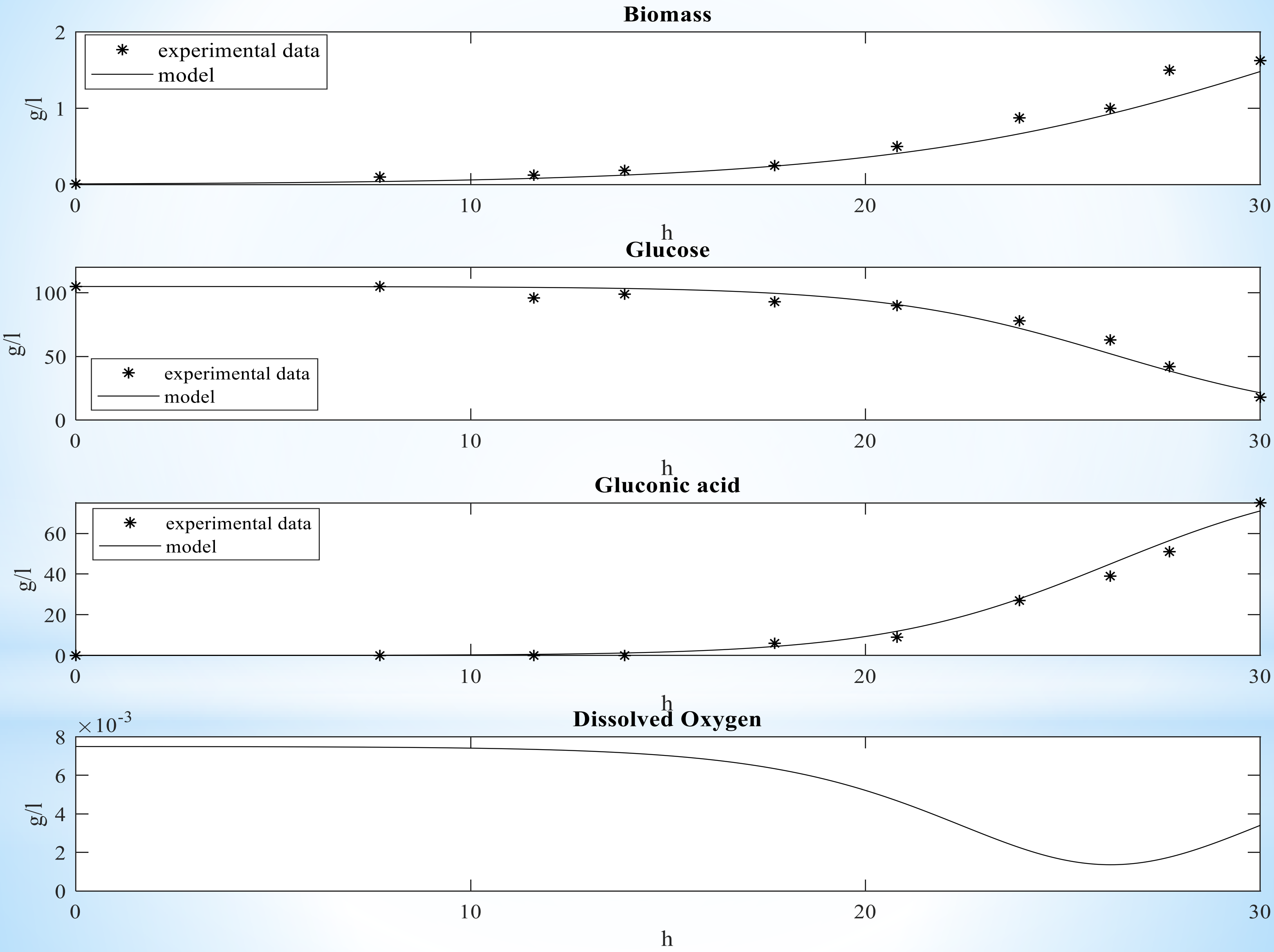
$$\frac{dX}{dt} = R_x;$$

$$\frac{dG}{dt} = -R_x - R_{GA};$$

$$\frac{dGA}{dt} = R_{GA};$$

$$\frac{dO}{dt} = -0.5R_{GA} + K_L a(O_2^{\ *} - O_2),$$

# Reducted model simulation

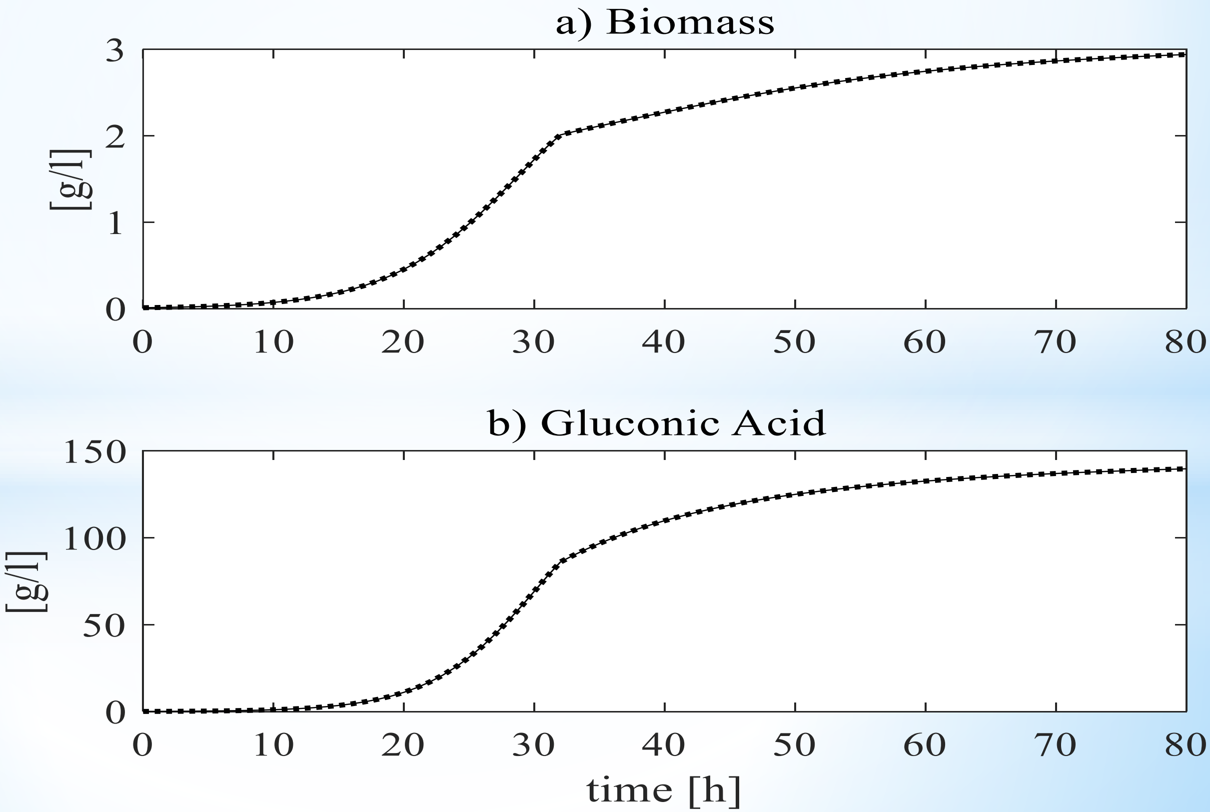


$$X_e = \frac{1}{k_1} Z_1 - \frac{k_2}{k_1 k_3}(Z_2 - O_2) - \frac{1}{k_1} G$$

$$GA_e = \frac{1}{k_3}(Z_2 - O_2)$$

$$\frac{dZ_1}{dt} = -DZ_1 + DG_{in}$$

$$\frac{dZ_2}{dt} = -DZ_2 + K_L a(O_2^* - O_2)$$



# Linear regression form of the control model

$$\frac{dX_e}{dt}/dt = X_e G\theta_1 - DX_e$$

$$\frac{dG}{dt} = -X_e G\theta_2 - GO_2\theta_3 - D(G - G_{in})$$

$$\frac{dO_2}{dt} = GO_2\theta_5 - DGA_e = -GO_2\theta_4 - DO_2 + K_L\alpha(O_2^* - O_2$$

$$\frac{dGA_e}{dt} = GO_2\theta_5 - DGA_e$$

# Continous Control of Glucose concentration

*First step*

$$\frac{dG}{dt} = -X_eG\theta_2 - GO_2\theta_3 - D(G - G_{in}))$$

*Second step*

$$\lambda(G^* - G) = dG/dt$$

*Third step*

$$D = \frac{-\lambda(G^*-G)-X_eG\theta_2-GO_2\theta_3}{G-G_{in}}$$

# Fed-Batch Control of Glucose concentration

*First step*

$$\frac{dG}{dt} = -X_eG\theta_2 - GO_2\theta_3 - \frac{F}{V}(G - G_{in})$$

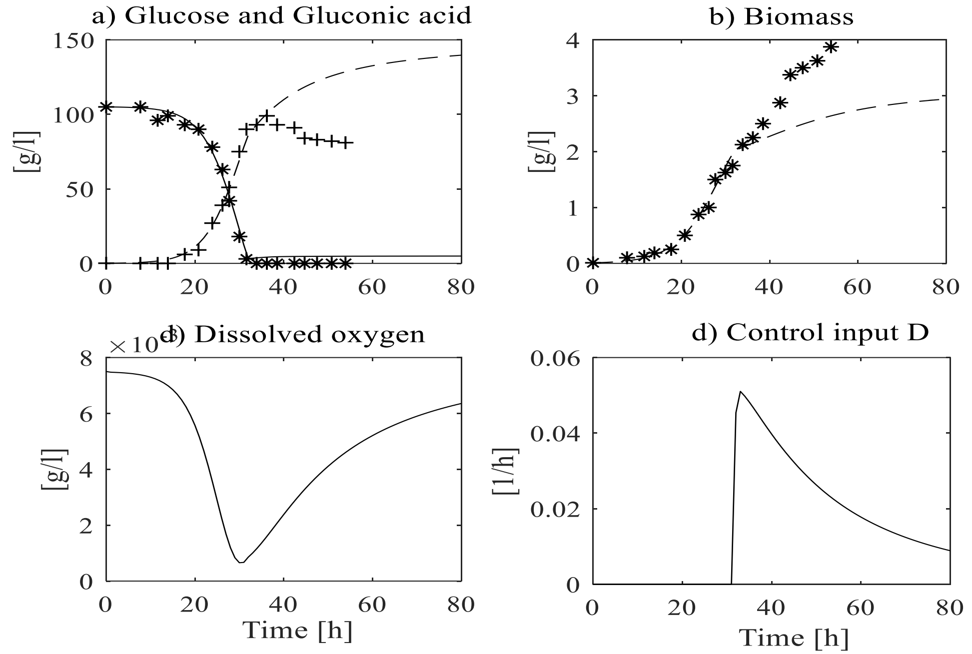
*Second step*

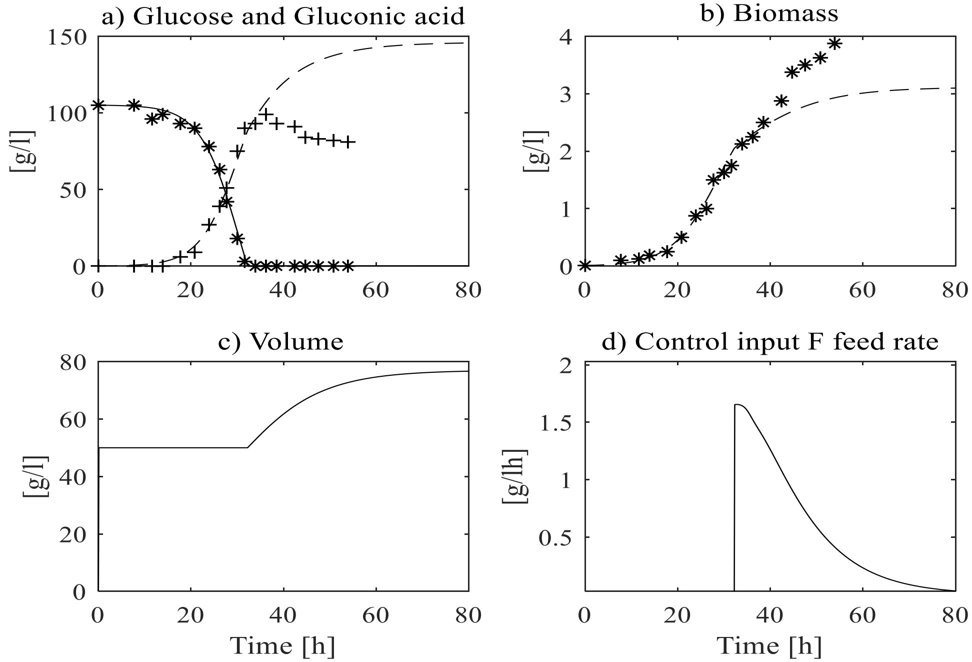
$$\lambda(G^* - G) = dG/dt$$

*Third step*

$$F = \frac{\boldsymbol{G_{in}}(-\lambda(G^*-G)-X_eG\theta_2-GO_2\theta_3)}{G-G_{in}}$$

$dV/dt=F$





Two approaches are considered. The first is to maintain some low value of the substrate (*G*) in the culture medium (0 or 3 g/l). Comparing the results obtained obtained until the eightieth hour of fermentation in sub-figures a, it was found that a fed-batch control achieves a higher concentration of the target product compared to the continuous control.

# Continous Control of Gluconic Acid Concentration

*First step* $$\frac{dGA_e}{dt} = GO_2\theta_5 - DGA_e$$

*Second step* $$\lambda(GA^* - GA_e) = dGA_e/dt$$

*Third step* $$D = \frac{-\lambda(GA^* - GA_e) + GO_2\theta_5}{GA_e}$$

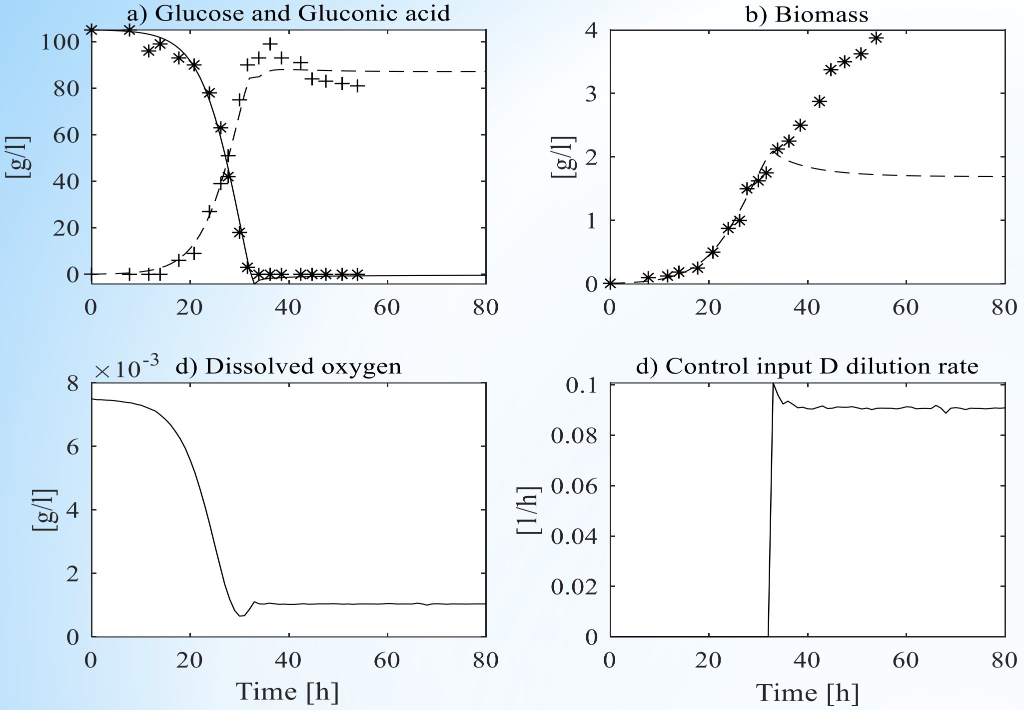
# Fed-Batch Control of Gluconic Acid Concentration

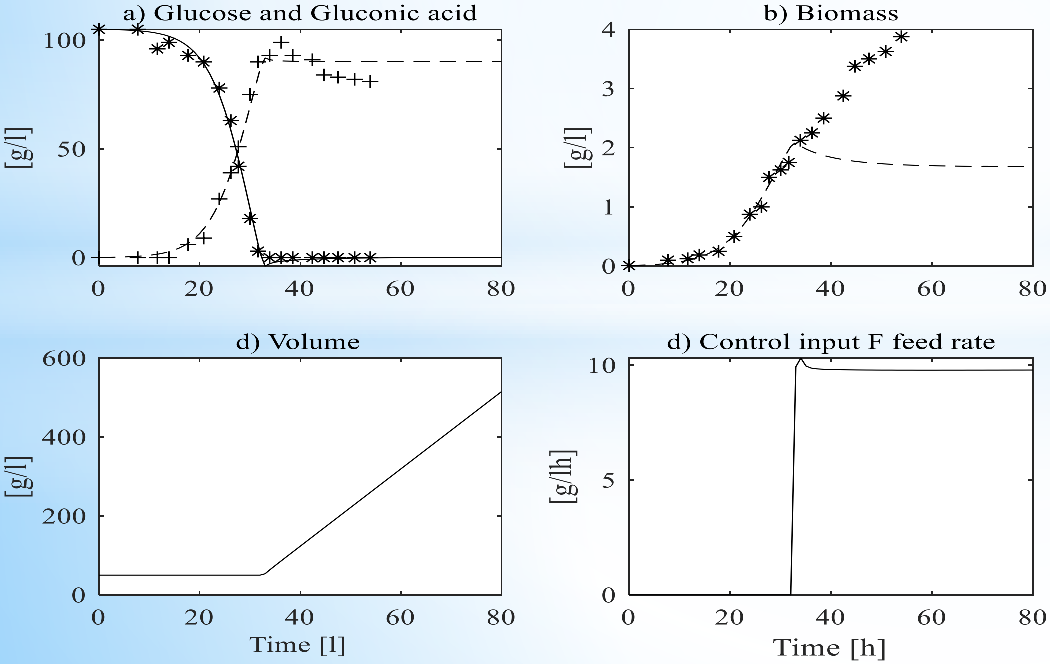
*First step* $$\frac{dGA_e}{dt} = GO_2\theta_5 - \frac{F}{G_{in}}GA_e$$

*Second step* $$\lambda(GA^* - GA_e) = dGA_e/dt$$

*Third step* $$F = \frac{V(\lambda(GA^* - GA_e) - GO_2\theta_5)}{GA_e}$$

$$dV/dt = F$$





In the second one, a constant concentration of the target product (*GA*), which corresponds to its maximum productivity is maintained. It should be noted that the fed-batch process must be stopped when the volume reaches the maximum working volume (80 l), while during continuous mode of cultivation, 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.

# Conclusions

The algorithms developed in this study will be integrated into the system together with similar algorithms, developed for other food production processes. The choice of a process as well as the activation of the functions related to the identification, monitoring and control will be realized by the users through simple actions. In this way, users will be able to get acquainted with the results of modern algorithms without being familiar with the theory of their development, as well as with the software of their implementation. What has been said so far gives grounds to define the system under developing InSEMCoBio as interactive and user-friendly.

# THANKS FOR YOUR ATTENTION