

Modelling of L-valine Repeated Fed-batch Fermentation Process Taking into Account the Dissolved Oxygen Tension

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Abstract: *This article deals with synthesis of dynamic unstructured model of variable volume fed-batch fermentation process with intensive droppings for L-valine production. The presented approach of the investigation includes the following main procedures: description of the process by generalized stoichiometric equations; preliminary data processing and calculation of specific rates for main kinetic variables; identification of the specific rates takes into account the dissolved oxygen tension; establishment and optimisation of dynamic model of the process; simulation researches. MATLAB is used as a research environment.*

Keywords: *Modelling, Optimisation, Repeated fed-batch process, Fed-batch process with droppings, L-valine.*

Introduction

At present the processes for microbial production of amino acids are among the most important in terms of tonnage and economical value. Market development has been particularly dynamic for the flavor-enhancer glutamic acid and the animal feed amino acids L-lysine, L-threonine, and L-tryptophan. Significant increase of the production of branched chain amino acids and all other amino acid is observed during the past decade [5]. Bioprocess technologies for their production were significantly improved by application of more sophisticated feeding and automation strategies [4].

The synthesis of mathematical models for biotechnological processes in principal is known to be the major task for the application of modern control science for their optimization. The models normally involve two kinds of parameters: the yield coefficients, which rely on the structure of the generalised stoichiometric reactions and the kinetic rates, which rely on the specific metabolism pathways [1].

Some approaches for dynamic modelling of L-valine fed-batch fermentation process have been described in our previous articles [2, 3] including modelling of fed-batch fermentation process with droppings. This article aims to present an approach for development of dynamic unstructured model for L-valine fed-batch fermentation process with intensive droppings of

the culture broth as well as investigation of the specificity of the process and its reflection on the obtained mathematical model. This approach presents extended variant of generalized stoichiometric equations takes into account the dropping conditions by sequential reactions. The specific rates: growth rate (μ), substrate utilization rate (ν), production rate (ρ) and dissolved oxygen utilization rate (γ) are estimated by optimisation procedure take into account the real – life experimental data.

Identification procedure, applied for estimation of the model structure and coefficients, taking in consideration the specificity concerning dropping procedure. The important stage of this procedure is the parametric optimisation of the model. The procedures for identification, optimisation and simulation researches are realized by **MATLAB** and **STAGRAPHICS** packages [6-9]. Main approaches and steps, used for development of mathematical models, are described in details in our previous articles [2, 3].

Materials and methods

Experimental procedures and analysis

The variable volume fed-batch fermentation process is carried out at laboratory scale fermentor with 7 l total volume. *Corynebacterium glutamicum* sp. B-023 is used as a producer. Analytical methods used for the characterisation of the process are as follows: biomass is measured as dry cell mass, [g·l⁻¹]; sugar concentration – as reducible compounds, [g·l⁻¹]; L-valine – by chromatographic method, [g·l⁻¹]. During the process on-line measurement of differed physical-chemical variables are done by proper sensors.

The experimental data are shown in Fig. 1. Dissolved oxygen concentration [mMol·l⁻¹] is denoted as DO in Fig. 1.

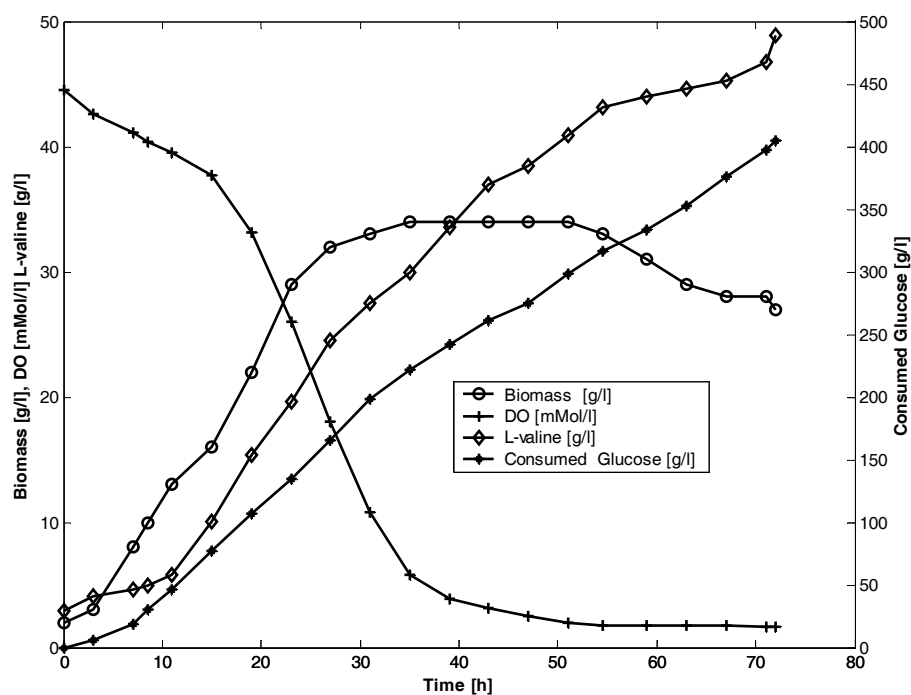


Fig. 1 Time course of the experimental data

Primary data processing

The primary data processing of the experimental data includes the following stages: transformation the different measurable units to unit [g·l⁻¹] taking into account changes (as strict balance) of the working volume during the process; calculation of the specific rates: growth rate (μ), [h⁻¹], substrate utilization rate (ν), [h⁻¹], production rate (ρ), [h⁻¹], dissolved oxygen utilization rate (γ), [h⁻¹]. To reach the final aim of primary data processing – calculation of the specific rates – the appropriate optimal approximation of the experimental data is done by a spline functions. The derivatives of variables describing the process kinetics are also calculated. The specific growth rate, the rate of substrate utilisation, the specific production rates are calculated by the equations:

$$\hat{\mu} = \frac{\dot{X}_t}{X_t}, \hat{\nu} = \frac{\dot{S}_C}{X_t}, \hat{\rho} = \frac{\dot{L}_{Vt}}{X_t}, \hat{\gamma} = \frac{\dot{C}}{X_t} \quad (1)$$

where: X_t – total biomass produced at proper time expressed as [g·l⁻¹], S_C – total sugar consumed at proper time expressed as [g·l⁻¹], L_{Vt} – total L-valine produced at proper time expressed as [g·l⁻¹], C – dissolved oxygen concentration expressed as [mMol·l⁻¹], and derivatives of these variables denotes as follows $\dot{X}_t, \dot{S}_C, \dot{L}_{Vt}, \dot{C}$. The total biomass produced (X_t) in calculation of the specific dissolved oxygen utilization rate (γ) is expressed as [mMol·l⁻¹].

The Eq. (1) presents an initial estimation of the values and shapes of specific rates. The specific rates must be calculated subject to initial and boundary conditions. These conditions are reflected to the inner behaviours of the kinetics variables.

The dynamic model subject to material balance of the process could be described as follows:

$$\frac{dX}{dt} = K_1 \hat{\mu} X - \frac{F_{IN}}{V} X - \frac{F_{OUT}}{V} X \quad (2)$$

$$\frac{dS_C}{dt} = K_2 \hat{\nu} X - \frac{F_{IN}}{V} S_C + \frac{F_{IN}}{V} S_{IN} - \frac{F_{OUT}}{V} S_C \quad (3)$$

$$\frac{dL_V}{dt} = K_3 \hat{\rho} X - \frac{F_{IN}}{V} L_V - \frac{F_{OUT}}{V} L_V \quad (4)$$

$$\frac{dC}{dt} = -K_4 \hat{\gamma} X + Q(t)(C^* - C) \quad (5)$$

$$\frac{dV}{dt} = F_{IN} - F_{OUT} \quad (6)$$

where: X – biomass concentration, [g·l⁻¹]; L_V – L-valine produced, [g·l⁻¹]; V – total volume, [l]; F_{IN} – feeding rate, [h⁻¹]; F_{OUT} – dropping rate, [h⁻¹]; C^* – initial oxygen saturation level; $Q(t)$ – function of the K_{La} coefficient and the time (t) and K_1, K_2, K_3, K_4 are yield coefficients.

The specific rate could be estimated by the non-linear least – squares optimization procedure based on experimental data and the initial values of the coefficients ($K_i, i = 1 \div 3$), and specific rates (μ, ν, ρ, γ). Note that in this optimization problem variable θ represents one of the

specific rates (μ, ν, ρ, γ) as a vector with components $\theta(t)^T = [\theta(t_0), \theta(t_1), \dots, \theta(t_f)]^T$, where $(t_0 - t_f)$ are moments of the taken samples. Also variable Y presents one of a pairs of the vectors $[X V]^T, [S_C V]^T, [L V]^T, C^T$ for each specific rate.

Suppose that for the specific growth rate (μ) , $[h^{-1}]$, substrate utilization rate (ν) , $[h^{-1}]$ and production rate (ρ) , $[h^{-1}]$ could be described

$$\frac{dY}{dt} = \mathbf{L}(\hat{K}_i, \hat{\theta})Y - D_L^{IN}Y - D_L^{OUT}Y \quad (7)$$

where $D_L^{IN} = \frac{F_{IN}}{V}$ – dilution level, $[h^{-1}]$; $D_L^{OUT} = \frac{F_{OUT}}{V}$ – dilution level as a result of the droppings, $[h^{-1}]$ and \mathbf{L} is a matrix as follows:

$$\mathbf{L}(\hat{K}_i, \hat{\theta}) = \begin{bmatrix} \hat{K}_i \hat{\theta} & 0 \\ 0 & 2 \frac{F_{IN}}{V} \end{bmatrix} \quad (8)$$

with notations for estimates of the parameters $\hat{K}_i, \hat{\theta}$.

Solution of the Eq. (7) is obtained by appropriate numerical method and could be describe as follows:

$$Y(K_i, \theta, t) = Y_0 + \int_{t_0}^{t_f} \left(\mathbf{L}(\hat{K}_i, \hat{\theta}) - \mathbf{I} D_L^{IN} - \mathbf{I} D_L^{OUT} \right) Y dt \quad (9)$$

where \mathbf{I} is a identity matrix.

The solution of the dissolved oxygen is

$$C(K_4, \gamma, t) = C_0 + \int_{t_0}^{t_f} (-K_4 \hat{\gamma} X - Q(t)C + Q(t) C^*) C dt \quad (10)$$

Suppose that the objective function for estimation of the specific rates is

$$F(K_i, \theta) = \min_{[K_i, \theta] \in R^n} \int_{t_0}^{t_f} (Y_{\text{exp}}(t) - Y(K_i, \theta, t))^2 dt \quad (11)$$

where Y_{exp} is a vector of the experimental data.

The result of this optimization problem is estimation of the parameters $\hat{K}_i, \hat{\theta}$.

The specific growth rate and the specific substrate utilisation rate are shown in Fig. 2 and Fig. 3.

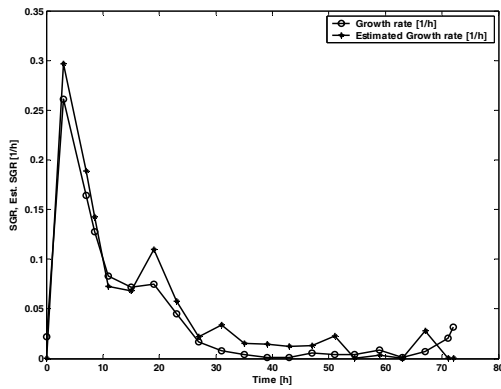


Fig. 2 Time course of the specific growth rates

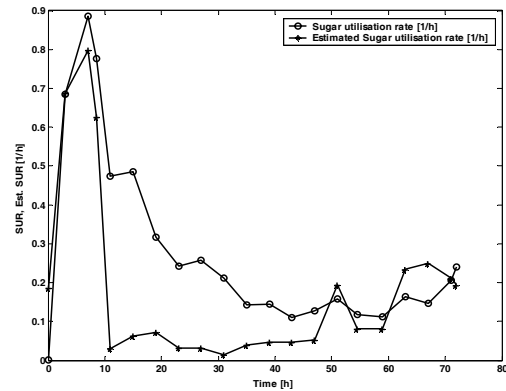


Fig. 3 Time course of the specific sugar utilisation rates

The notations are: SGR – specific growth rates, Est. SGR – specific growth rate estimated by optimization. SUR – specific sugar utilisation rate, Est. SUR – specific sugar utilisation rates estimated by optimisation.

The specific production rate and dissolved oxygen utilisation rate are shown in Figs. 4 and 5 respectively.

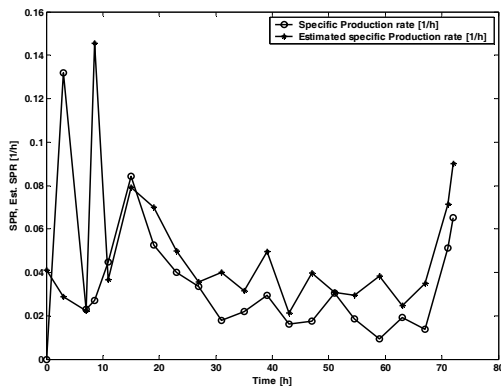


Fig. 4 Time course of the specific production rate

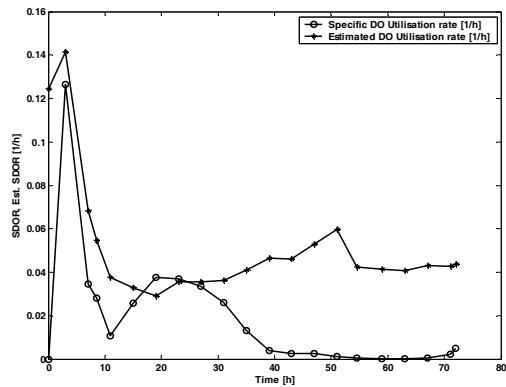


Fig. 5 Time course of the specific DO utilisation rate

The notations are: SPR – specific production rate, Est. SPR – specific production rate estimated by optimisation, SDOR – specific DO utilisation rate, Est. SDOR – specific DO utilisation rate estimated by optimisation.

Results and discussion

The identification procedure includes the following main stages: determination of the set of generalized stoichiometric equations; identification of the specific rates; description of the dynamic model subject to dropping conditions; parametric optimisation of the obtained model and simulation.

Generalized stoichiometric equations

The fermentation processes could be described by the following scheme of generalized stoichiometric equations:



where: $\varphi_X, \varphi_G, \varphi_S, \varphi_{LV}, \varphi_F, \varphi_{OUT}$ are rates of the generalized stoichiometric reactions, $[\text{g}\cdot\text{l}^{-1}\cdot\text{h}^{-1}]$, V_0 – initial volume of the culture broth, V_f – final volume of the culture broth, $[\text{l}]$; X – biomass concentration, $[\text{g}\cdot\text{l}^{-1}]$; S – substrate concentration as a sugar remain concentration – S_R or sugar consumed concentration – S_C , $[\text{g}\cdot\text{l}^{-1}]$; L_V – L-valine concentration, $[\text{g}\cdot\text{l}^{-1}]$; C – dissolved oxygen concentration (DO), $[\text{mMol}\cdot\text{l}^{-1}]$.

The hypotheses concerning the specific rates of the amino acids biosynthesis are utilised as follows:

$$\hat{\mu} = \hat{\mu}(S_C, C), \hat{v} = \hat{v}(\hat{\mu}, X), \hat{\rho} = \hat{\rho}(\hat{\mu}, X), \hat{\gamma} = \hat{\gamma}(\hat{\mu}, X, C) \tag{13}$$

The proposed hypotheses are discussed below. It could be seen (Fig. 2 – Fig. 5) that some of the fundamental kinetic relations, for example Monod law, are not performed for the obtained specific rates. On the other hand the fundamental relations such a Monod law reveals the approach to reach new relations. The Monod law has the following properties: hidden linear structure (the reciprocal transformation reveals this structure); hidden exponential structure if the Monod law is divided by the substrate concentration (S) the result reveals Pade' approximant of the function $\exp(-K_S/S)$. The second hidden property of the Monod law gives an argument for the exponential structure investigation of the specific rates under hypotheses (13).

Dynamic model of the fed-batch process with dropping

The dynamic model subject to material balance of the process could be describe as follows

$$\begin{aligned}
 \frac{d\xi}{dt} &= \mathbf{K}\varphi(\xi) - D_L^{IN}\xi + F - D_L^{OUT}\xi \\
 y &= \mathbf{P}\xi
 \end{aligned} \tag{14}$$

where $\xi^T = [X, S_R, S_C, L_V, C, V]^T$ – state space vector; $\varphi^T = [\varphi_X, \varphi_G, \varphi_S, \varphi_{LV}, \varphi_F, \varphi_{OUT}]^T$ – vector of reaction's rates; $F^T = [0, 0, f_1, 0, f_2, 0]^T$ – vector of input streams with elements $f_1 = D_L^{IN}S_{IN}$ and $f_2 = Q(t)(C^* - C) + (D_L^{IN} + D_L^{OUT})C$; \mathbf{P} – matrix; y – measurable output from sensors; S_{IN} – concentration of the feeding solution, $[\text{g}\cdot\text{l}^{-1}]$, \mathbf{K} – matrix of yield coefficients as follows:

$$\mathbf{K} = \begin{bmatrix} k_{11} & k_{12} & 0 & -k_{14} & 0 & \pm k_{16} \\ 0 & 0 & -k_{23} & 0 & 0 & 0 \\ -k_{31} & -k_{32} & k_{33} & -k_{34} & 0 & \pm k_{36} \\ 0 & 0 & 0 & k_{44} & 0 & \pm k_{46} \\ 0 & -k_{52} & 0 & -k_{54} & 0 & 0 \\ 0 & 0 & 0 & 0 & k_{65} & \pm k_{66} \end{bmatrix}, \mathbf{P} = \begin{bmatrix} 0 & 0 & 0 & 0 & 1 & 0 \\ 0 & 0 & 0 & 0 & 0 & 1 \end{bmatrix}$$

Identification procedure includes the following stages:

- Model structure identification.
- Estimation of the coefficients, which is expressed as yield and reaction rate coefficients, as a real numbers.

The model structure identification is obtained by reduction of the state space vector by one variable S_R and the following substitutions:

$$\begin{aligned} k_{11}\varphi_X + k_{12}\varphi_G - k_{14}\varphi_{L_v} &= K_1\mu(S, C)X; k_{23} = 0 \\ -k_{31}\varphi_X - k_{32}\varphi_G + k_{33}\varphi_S - k_{34}\varphi_{L_v} &= K_2\nu(\mu, X)X \\ k_{44}\varphi_{L_v} &= K_3\rho(\mu, X)X \\ -k_{52}\varphi_G - k_{54}\varphi_{L_v} &= -K_4\gamma(\mu, X, C)X \\ k_{65}\varphi_F &= 2D_L^{IN}V \end{aligned} \quad (15)$$

The rates of the generalized stoichiometric reactions are reviled by the specific rates as it is shown by the equalities (15). The models of the specific rates are discussed below.

Dropping conditions

It is assumed that at the discrete time moments of the dropping the derivatives of the kinetic variables are equal to zero. The dropping conditions subject to model (2) – (6) are satisfied as follows:

- Dropping conditions for growth

$$F_{OUT} = K_1\mu(t_k)V(t_k) - F_{IN} \quad (16)$$

- Dropping conditions for L-lysine production

$$F_{OUT} = K_3\rho \frac{X(t_k)}{L(t_k)}V(t_k) - F_{IN} \quad (17)$$

- Dropping conditions for substrate utilization

$$F_{OUT} = K_2\nu \frac{X(t_k)}{S(t_k)}V(t_k) + F_{IN} \left(\frac{S_{IN} - S_C(t_k)}{S_C(t_k)} \right) \quad (18)$$

The comparison of the expressions (17) – (18) obtains the equality

$$K_3 \rho \frac{X(t_k)}{L(t_k)} = K_2 v \left(\frac{X(t_k)}{S_C(t_k)} \right) + \frac{F_{IN}}{V} \left(\frac{S_{IN}}{S_C(t_k)} \right) \quad (19)$$

It could be emphasized that these conditions are satisfied at the discrete time moments.

Models of the specific rates

The identification procedure of the specific rates includes the following steps:

- Application of linear regression for selection of suitable set of predictors and initial estimates of the parameters.
- Non-linear regression application for final parameter estimation.
- Simulation in MATLAB environmental (Figs. 6 – 9).

The results of the identification procedure are derived by **STATGRAPHICS** facilities as follows.

Table 1. Models of the specific rates

Model	R^2
$\hat{\mu} = \exp \left(\begin{aligned} &a_0 + a_1 S_C + a_2 S_C^2 + a_3 C + a_4 C^2 + a_5 C^3 + a_6 (S_C C^2) + \\ &+ a_7 (S_C^2 C) + a_8 (S_C C^3) + a_9 (S_C^3 C) \end{aligned} \right)$	$R^2 = 0.74$ (20)
$\hat{v} = \exp(b_0 + b_1 \mu + b_2 \mu^2 + b_3 \mu^3 + b_4 X + b_5 X^2 + b_6 X^3 + b_7 X^4)$	$R^2 = 0.80$ (21)
$\hat{\rho} = \exp \left(\begin{aligned} &c_0 + c_1 \mu + c_2 \mu^2 + c_3 \mu^3 + c_4 \mu^4 + c_5 X + c_6 X^2 + c_7 X^3 + \\ &+ c_8 X^4 + c_9 (\mu X^2) + c_{10} (\mu^2 X) \end{aligned} \right)$	$R^2 = 0.995$ (22)
$\hat{\gamma} = \exp(f_0 + f_1 \mu + f_2 \mu^2 + f_3 \mu^3 + f_4 X + f_5 X^2 + f_6 X^3 + f_7 C + f_8 C^2 + f_9 C^3)$	$R^2 = 0.991$ (23)

The acceptance of the specific rate models is based on the value of the determination coefficient (R^2) obtained by non-linear regression. Investigation of the residuals shows that the majority parts of them are normally distributed but the serial correlation could be seen.

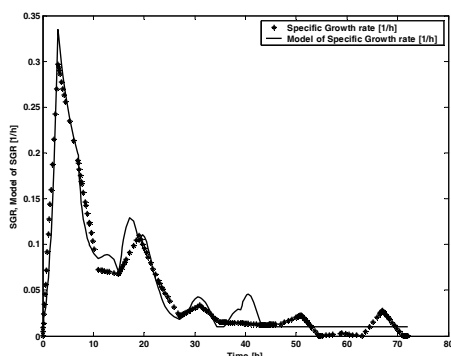


Fig. 6 Time course of the specific growth rate

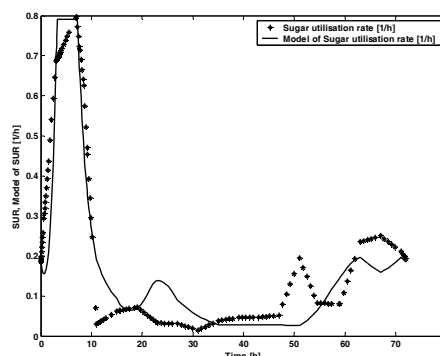


Fig. 7 Time course of the specific substrate utilisation rate

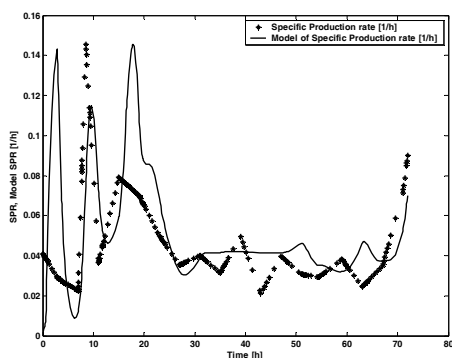


Fig. 8 Time course of the specific production rate

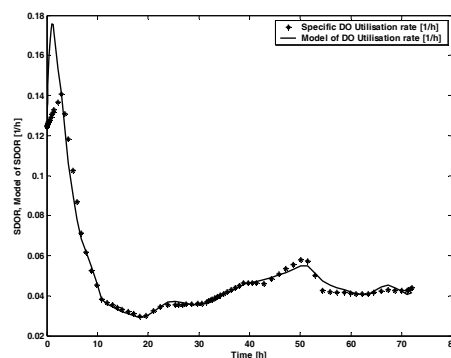


Fig. 9 Time course of the specific DO utilisation rate

Optimization and simulation

The second stage of this procedure is connected with the parametric optimization of the model. The Levenberg-Marquardt and trust-region algorithms with least squares objective function are used for optimization. The results are shown in Table 2.

Table 2. Estimated parameters

$K_1 =$	1.0999987654	$K_3 =$	0.8763091125
$a_0 =$	-945.6313156906	$c_0 =$	-0.1573747250
$a_1 =$	6.8934528346	$c_1 =$	-139.9591620725
$a_2 =$	-0.0126724764	$c_2 =$	3438.7037377048
$a_3 =$	2035.6832209990	$c_3 =$	-16288.1457419416
$a_4 =$	-1094.4874073790	$c_4 =$	23118.3024186340
$a_5 =$	-9.7524792850	$c_5 =$	-2.0049940020
$a_6 =$	-0.3824799380	$c_6 =$	0.2306981150
$a_7 =$	0.0153188510	$c_7 =$	-0.0087843638
$a_8 =$	2.8974402983	$c_8 =$	0.0001077039
$a_9 =$	$-1.9005626754 \times 10^{-5}$	$c_9 =$	0.0943875759
$K_2 =$	0.5500000000	$c_{10} =$	-55.3316752540
$b_0 =$	-4.0056815196	$K_4 =$	3.7963175785
$b_1 =$	-9.3625487787	$f_0 =$	2.9473564641
$b_2 =$	60.8258709465	$f_1 =$	5.6083794871
$b_3 =$	-77.8933745419	$f_2 =$	-44.8301170577
$b_4 =$	1.5038299288	$f_3 =$	76.1872394356
$b_5 =$	-0.1763699123	$f_4 =$	-0.0676848582
$b_6 =$	0.0073331117	$f_5 =$	$9.6803613194 \times 10^{-5}$
$b_7 =$	-0.0001077569	$f_6 =$	$5.9881726627 \times 10^{-7}$
		$f_7 =$	-0.1369818332
		$f_8 =$	0.0095522004
		$f_9 =$	-0.0001947541

During the parametric optimization the experimentally established optimal modes of the feeding rate and oxygen saturation are applied. The concentration S_{IN} as a function is also obtained by an optimization based on the experimental data. The results are shown below (Fig. 10).

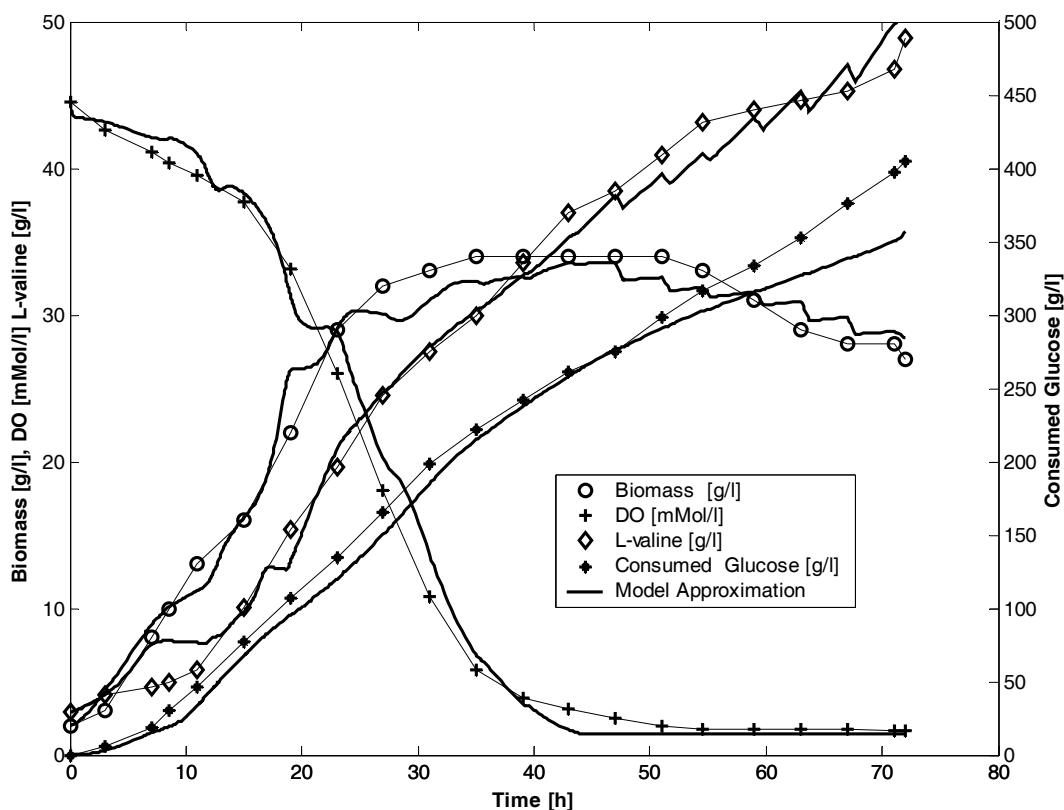


Fig. 10 Time course of the experimental data and model for main kinetic variables

Conclusions

The following consideration and conclusions can be derived from the investigation, connected with development of mathematical model of the discussed process.

1. The trend and values of the specific rates are estimated based on the experimental data and material balance followed by additional data processing by optimization procedure (see Figs. 2 – 5).
2. The models of the specific rates are obtained by linear and non-linear regression. These procedures permit investigation of the model structure and estimation of the initial parameters. Additional simulation shows the properties of the derived models (see Figs. 6 – 9)
3. The final stage of the investigation is connected with the parametric optimisation of the model through the non-linear optimisation procedure under the confidence intervals of the parameters using Optimisation Toolbox. The Levenberg-Maquardt and trust-region algorithms with least squares objective function are used for optimisation.
4. The mathematical model describes the trend of the investigated experimental data and specific rates of the main kinetic variables.

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