Applicability of the Three Dimensional Growth Model in Description of *Mucor miehei NCAIM 5238* Cultivation and Renin Biosynthesis

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Original scientific paper
Received: 1. 4. 2001..
Accepted: 1. 7. 2001.

In memoriam to Prof. Emeritus Vera Johanides

Fungus *Mucor miehei NCAIM 5238* during its growth in complex nutritive media effectively produces proteolytic enzymes, which besides hydrolitic also exhibit coagulation effects. Therefore, it is applied in industrial production of complex enzymatic preparation (renin), which is used in cheese production. The kinetics of microorganism growth and renin production were studied in a pilot-plant bioreactor of 1200 L working volume. During the batch culture the microorganism was growing in filaments and pellets, the later becoming predominant at the end of batch culture.

The present work is based on experimental evidence, which indicates that the process kinetics in general could be roughly described by applying a simple mathematical model based on the three dimensional growth concept. The applicability of the model with respect to biomass growth, substrate uptake, oxygen transfer and uptake as well as the product formation kinetics was investigated.

The batch culture range after 10 hours of the apparent lag phase was subjected to the study with respect to the mathematical model application. Application of the minimum variance method yielded kinetic parameters which gave a close fit between experimental and simulated data (coefficients of determination are $R^2_x = 0.985$; $R^2_s = 0.995$; $R^2_{DO} = 0.981$; $R^2_P = 0.975$). Computer simulation of the combination of batch and fed batch operations indicated a possible increase in renin production up to 30%.

Keywords:
Renin, *Mucor miehei*, mathematical modeling

Introduction

Fungus *Mucor miehei* NCAIM 5238 during its growth in complex nutritive media effectively produces proteolytic enzymes, which besides hydrolitic also exhibit coagulation effects.[1–3] It is applied in industrial production of complex enzymatic preparation (renin), which is used in cheese production.[4] Since the quality and price of cheese depend on quality and price of renin based additives there is economic incentive to optimise renin production.[5–9] Optimisation of renin production can be greatly improved by applying knowledge of process principles. A modern approach to process optimisation is based on the use of mathematical models of process kinetics derived from experimental data. Models must be verified by investigation of variance between model predictions and experimental data. In the case of biotechnological processes the modelling should account for numerous variables and unknown interactions which influence the process. In view of great uncertainties due to modelling biological complexities, simple and robust models are developed with an aim to assist process optimisation. Such simplified models must reflect the main interactions and obey the biological and physical laws.

During experimental investigation of the kinetics of renin biosynthesis in a bioreactor of 1200 L working volume, it was observed that microorganism grew in filaments and with forming pellets which gradually became dominant growth form during batch operation.[10] During microorganism growth nutrients are consumed and proteolytic enzymes (renin) are accumulated. A typical duration of a batch operation is about 70 h, followed by process of the enzyme extraction from fermentation broth.[10] Efficacy of this process is greatly dependent on properties of fermentation media. Experimental data applied in this work are provided from the work of Šlaus.[10] All data are those collected during the batch phase of operation.

Mathematical model

Based on experimental data, a simple unstructured mathematical model based on kinetic rates developed for three dimensional growth of mycelia was choosen to be applied here. It has been previously proven by Bošnjak[11,12] as a successful model for description of secondary metabolite production by mycelia. The model is expressed as a set of nonlinear differential equations accounting for mass balances.

The model for biomass growth is given by:

$$\frac{\mathrm{d}}{\mathrm{d}t}\gamma_{\mathrm{X}} = k_1 \cdot \gamma_{\mathrm{X}}^{2/3} - k_2 \cdot \gamma_{\mathrm{X}} - D \cdot \gamma_{\mathrm{X}} \qquad (1)$$

Mass balance for substrate consumption:

$$\frac{\mathrm{d}}{\mathrm{d}t}\gamma_{\mathrm{S}} = -k_{\mathrm{sub}} \cdot \frac{\gamma_{\mathrm{S}}}{K_{\mathrm{S}} + \gamma_{\mathrm{S}}} \cdot \gamma_{\mathrm{X}} + D \cdot (\gamma_{\mathrm{S0}} - \gamma_{\mathrm{S}}) \qquad (2)$$

Mass balance for dissolved oxygen transfer and consumption is (supposing oxygen uptake rate is proportional to substrate uptake rate):

$$\frac{\mathrm{d}}{\mathrm{d}t}\gamma_{\mathrm{D0}} = k_1 a \cdot (\gamma_{\mathrm{DO}}^{*} - \gamma_{\mathrm{DO}}) - q_{\mathrm{OX}} \cdot \frac{\gamma_{\mathrm{S}}}{K_{\mathrm{S}} + \gamma_{\mathrm{S}}} \cdot \gamma_{\mathrm{X}} + D \cdot (\gamma_{\mathrm{DOS0}} - \gamma_{\mathrm{DO}}) \qquad (3)$$

Balance for product synthesis is given by:

$$\frac{\mathrm{d}}{\mathrm{d}t}\gamma_{\mathrm{P}} = k_{\mathrm{P}} \cdot \gamma_{\mathrm{X}} - D \cdot \gamma_{\mathrm{P}} \qquad (4)$$

Alternative product synthesis kinetics is based on growth dependent product synthesis with according product autolysis and product competitive inhibition effects, as expressed by equations:

$$\frac{\mathrm{d}}{\mathrm{d}t}\gamma_{\mathrm{P}} = k_{\mathrm{pm}} \cdot \frac{\gamma_{\mathrm{S}}}{K_{\mathrm{S}}^{*} \cdot \alpha_1 + \gamma_{\mathrm{S}}} \cdot \gamma_{\mathrm{X}} - k_{\mathrm{D}} \cdot \gamma_{\mathrm{P}} - D \cdot \gamma_{\mathrm{P}} \qquad (5)$$

and

$$\alpha_1 = 1 + \beta_i \cdot \gamma_{\mathrm{P}} \qquad (5a)$$

where β_i determines effect of product inhibition .

During continuous operation the dilution rate is constant and given by:

$$D_{\mathrm{CC}} = \frac{q}{V_{\mathrm{C}}} \qquad (6)$$

For fed batch operation at constant feeding flow rate the dilution rate is time dependent and is given by:

$$D_{\mathrm{FBC}} = \frac{v}{V_{\mathrm{L}} + v \cdot t} \qquad (7)$$

In the dissolved oxygen balance (3) a concentration of oxygen dissolved in nutrient stream is assumed at saturation level:

$$\gamma_{\mathrm{SODO}} = \gamma_{\mathrm{DO}}^{*} \qquad (8)$$

Commonly, during fed batch production, when optimal conditions for biosynthesis prevail, the effect of product degradation k_{d} is small, and can be negligible in model simulation.

Estimation of kinetic parameters and computer simulation

Kinetic parameters are estimated by minimisation of variance between numerical solutions of the differential balance (1–5) and experimental data. For numerical integration of stiff differential equations the numerical algorithm LSODE provided as *NDSolve* program in Wolfram Research *Mathematica*[13] is applied. The variance is minimised by iterative procedure of the steepest descent with numerical evaluation of gradients. Minimum of variance is evaluated by use of *FindMinimum* from Wolfram Research *Mathematica*.[13] The residual errors between the model and experimental data are evaluated by coefficients of determination. Derived model with estimated parameters is applied for process simulation with the objective to improve production by combination of fed batch and batch modes of bioreactor operation.

Results and discussion

Results of estimation of kinetic parameters are given in Table 1 whereas coefficients of determination for concentrations are given in Table 2. Experimental results obtained during 80 hours of batch production are given in Fig. 1- 4. In the same figures, results of computer simulation of the model equations (1–4) with the estimated parameters are plotted. The model predictions are in a close agreement with experimental data during the whole period of production, i.e. for the region of exponential growth (first 40 h) and the substrate limitation (last 20 h). Statistical measure of the model validation can be affirmed by the coeffi-

Table 1 – *Model parameters*

Parameter	Value	Remark
k_1	0.76	Fig. 1, 5a.
k_1	0.85	Fig. 6a.
k_2	0.226	Fig. 1, 5a, 6a.
k_{sub}	0.051	Fig. 2, 5b, 6b.
$k_l a$	158.0	Fig. 4, 6d.
q_{ox}	218.0	Fig. 4, 6d.
k_{pm}	3.15	Fig. 3, model eq. 4.
K_s	22.57	Fig. 2, 5b, 6b.
k_d	0.01	Fig. 3, model eq. 5
K^*_s	5.0	Fig. 3, model eq. 5
β_i	2.0 10^{-6}	Fig. 3, model eq. 5

Table 2 – *Determination coefficients between experimental data and model predictions for: biomass, substrate, dissolved oxygen, and product concentrations.*

Deter. coef.	Value	Remark
R^2_X	0.985	Fig. 1.
R^2_S	0.995	Fig. 2.
R^2_{DO}	0.981	Fig. 4
R^2_P	0.975	Fig. 3, model eq. 5
R^2_P	0.945	Fig. 3, model eq. 4

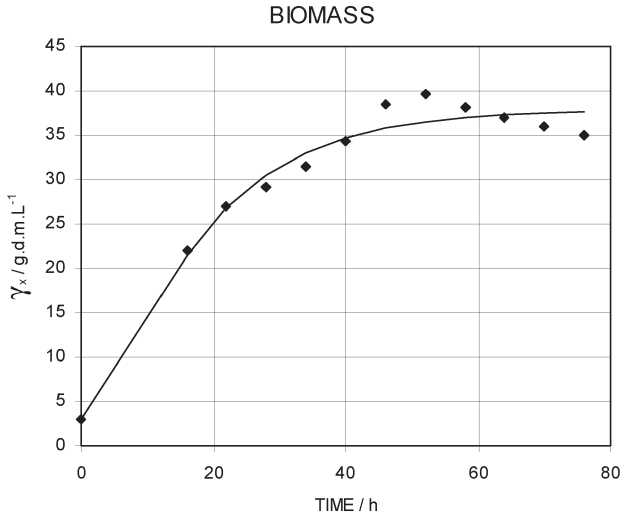


Fig. 1 – *Experimental BC data for biomass concentration (◆) and model predictions (–)*

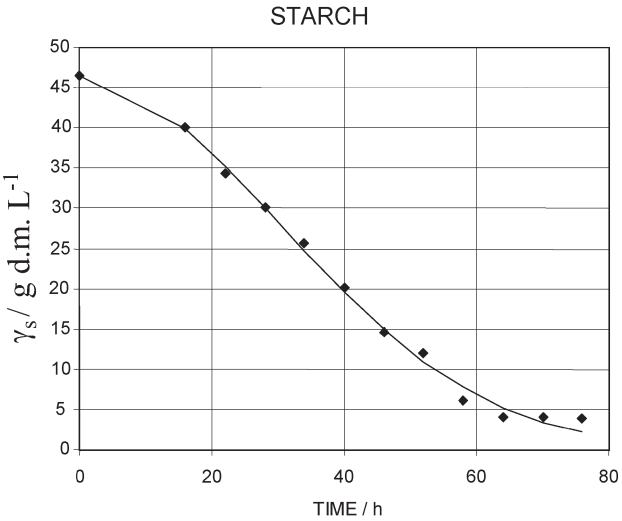


Fig. 2 – *Experimental BC data for starch concentration (◆) and model predictions (–)*

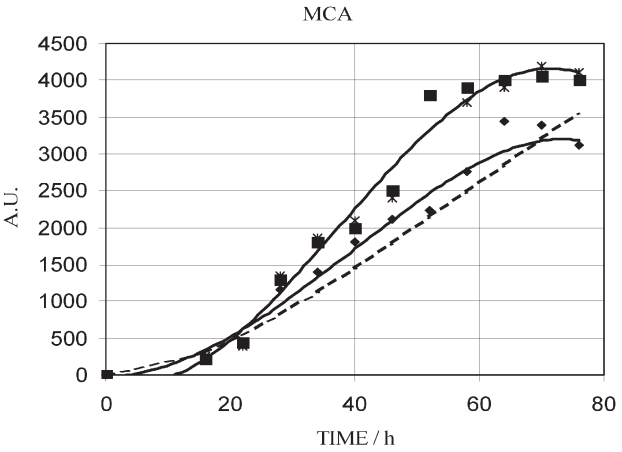


Fig. 3 – *Experimental BC data for renin enzymatic activity determined by different analytical methods (method 1 ◆; method 2 ■), and predictions by two models (model eq. 4 depicted by – –, model eq. 5 depicted by ——).*

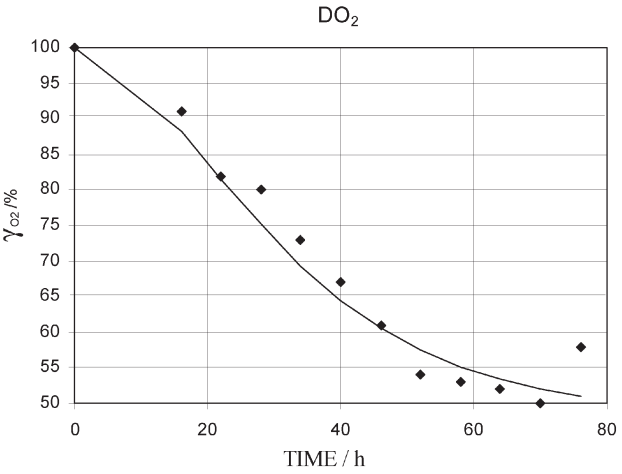


Fig. 4 – *Experimental BC data for dissolved oxygen concentration (◆) and model predictions (–)*

cients of determination (Table 2.). The coefficients have high values, in the range from 0.975 to 0.995. The highest agreement is obtained for the substrate concentration, while the least agreement is observed for the product. The product balance (5) has given better agreement with experimental data when it was compared to the simplified form (4), i.e. when constant specific rate of product synthesis was assumed. Results for renin concentration (activity)[10] during an analogue experiment are presented in Fig. 2. Experimental values are depicted by symbols representing two different analytical methods for determination of renin activity. Although the different experimental methods result in slightly different values of concentrations (activities), the same mathematical model (different k_p values) can predict the both results, if the specific rate constant is accounted by minimisation of the variance.

Predictions of fed batch production

Based on a good agreement between experimental data and the mathematical model (1–5), the model equations can be used in a computer simulation of a fed batch production with the aim to optimise productivity of renin. As shown in Fig. 5–6 two cases of improved productivity based on the combination of batch and fed batch operation are demonstrated. The production is simulated for batch operation for the first 50 h of operation, followed by fed batch in the time interval from 50 to 80 h, and continued as batch for the period from 80 to 100 h. The fed batch interval starts at 80 % of the total reactor volume and is conducted at constant feed rate up to 100 % of reactor working volume. Applied are constant concentration of starch in balanced feed of γ_{S0} = 60 g L^{-1}, and γ_{S0} = 200 g L^{-1}, presented in Fig 5 and 6 respectively.

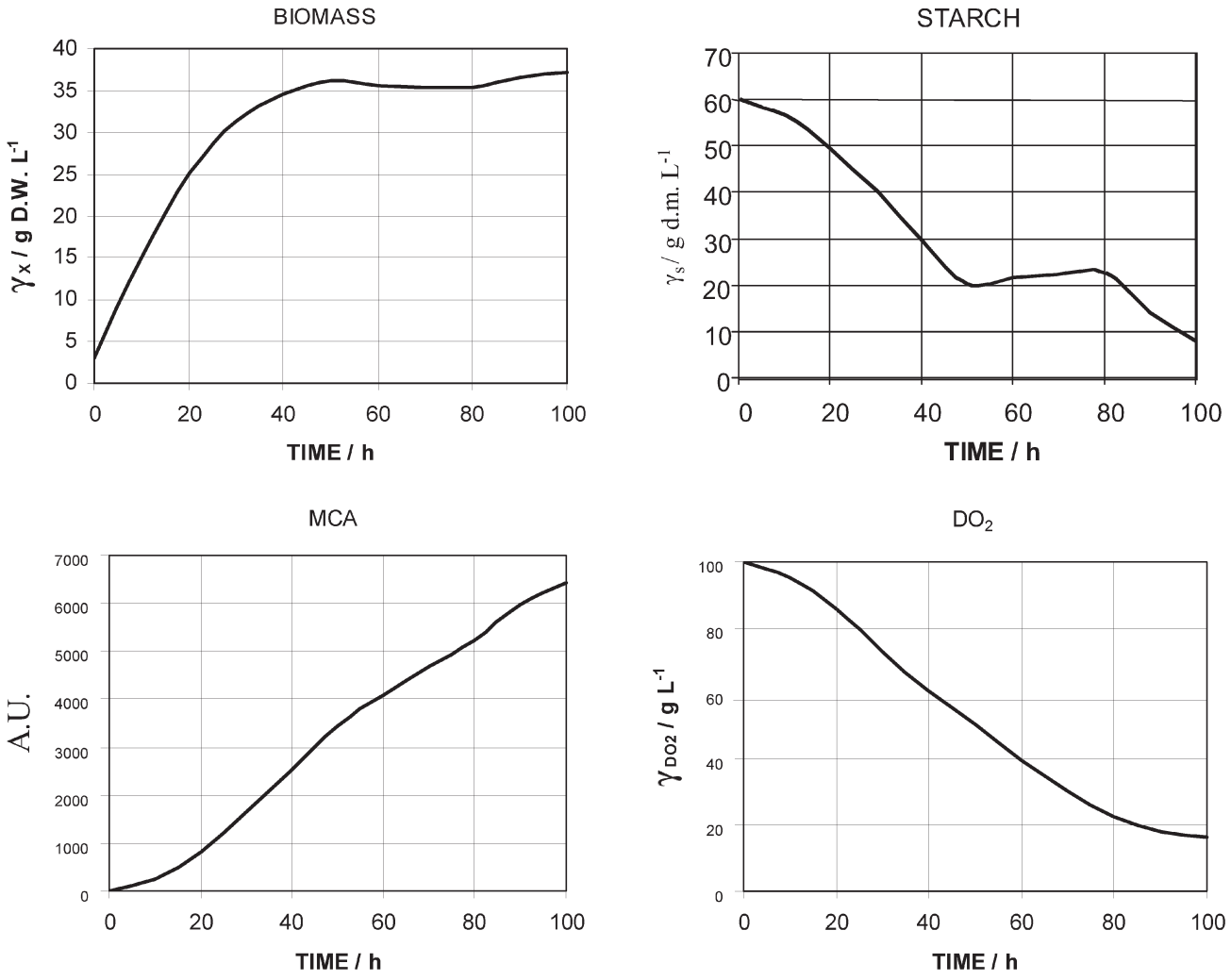
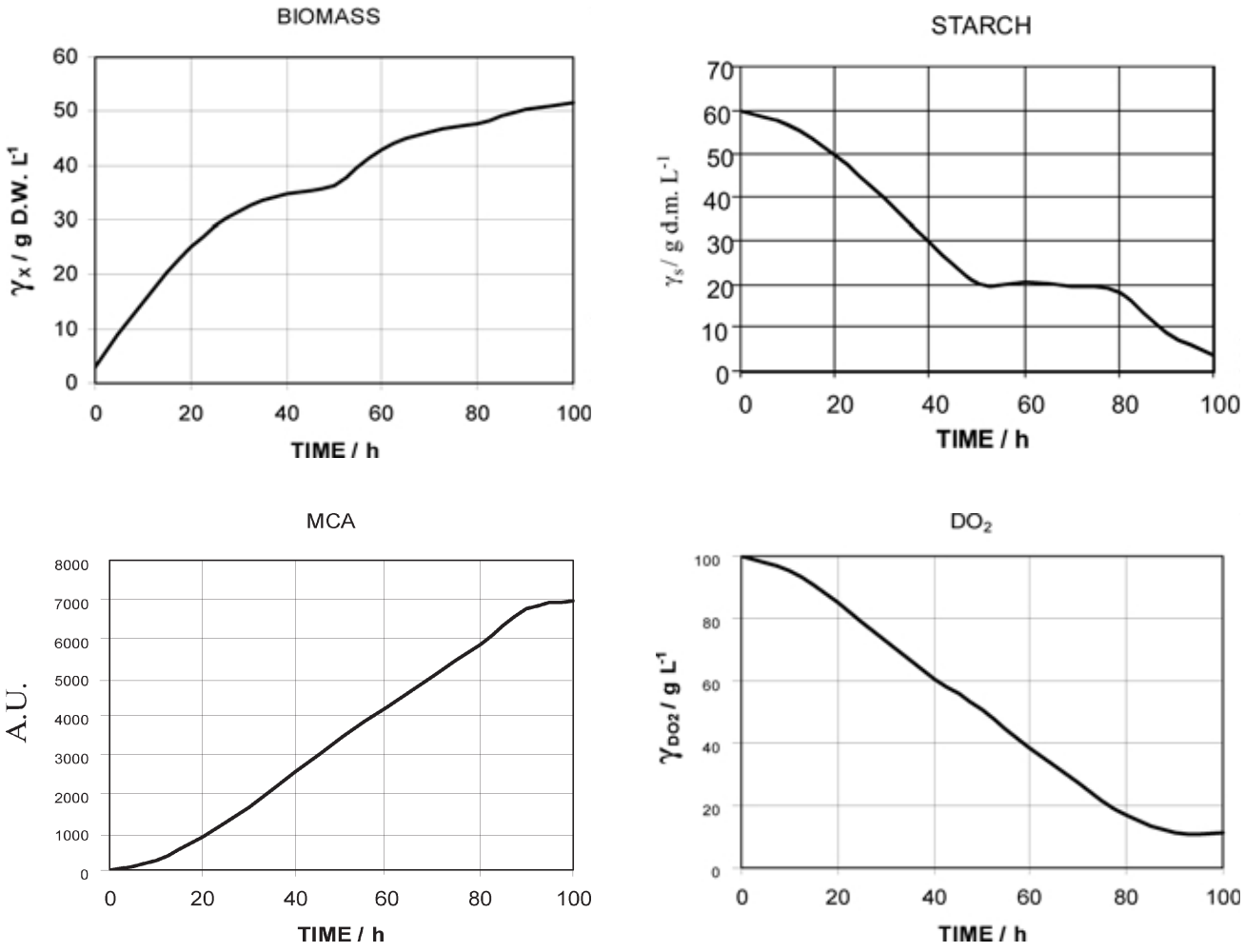


Fig. 5 – *Model predictions of biomass concentration (5a), starch concentration (5b), the enzymatic activity (5c), and dissolved oxygen concentration (5d) obtained by batch operation during the first 50 h, followed by fed batch during the interval 50 to 80 h, continued with batch operation till 100 h. During the fed-batch interval volumetric substrate flow rate is v = 10 L/h, V_L = 1200 L, and the substrate concentration in feed is γ_{SO} = 60 g/L*



F i g . 6 – *Model predictions of biomass concentration (6a), starch concentration (6b), the enzymatic activity (6c), and dissolved oxygen concentration (6d) obtained by batch operation during the first 50 h, followed by fed batch during the interval 50 to 80 h, continued with batch operation till 100 h. During the fed-batch interval volumetric substrate flow rate is v = 10 L/h, V_L = 1200 L, and the substrate concentration in feed is γ_{SO} = 200 g/L. Upon the fed-batch impulse the specific three-dimensional growth rate constant is increased to 0.85 $g^{1/3}$ $L^{-1/3}$ h^{-1}.*

The simulation results indicate a possible increase in renin productivity of 20 to 30 % compared to batch process.

Conclusions

Based on three dimensional model of biomass growth a mathematical model for prediction of biomass, substrate, product (renin), and dissolved oxygen is developed.

The model parameters are estimated from minimisation of variance between experimental data and predictions by numerical solutions of the differential model equations. There is obtained a high degree of agreement between experimental data and the model predictions. The model and experimental data have degrees of determination between 0.97 and 0.99.

Improvement of process productivity from 20 to 30 % is predicted by use of the combination of batch and fed batch operation.

List of Symbols

A. U. – arbitrary units
D – dilution rate, h^{-1}
D_{CC} – dilution rate during continuous phase of production, h^{-1}
D_{FBC} – dilution rate during fed batch phase of production, h^{-1}
d. m. – dry mass, g
k_1 – specific rate constant for three dimensional growth, $g^{1/3}$ $L^{-1/3}$ h^{-1}
k_2 – specific rate constant for biomass autolysis, h^{-1}
$k_l a$ – volumetric oxygen transfer rate, h^{-1}
k_P – specific rate of product synthesis, h^{-1}
k_{pm} – maximum specific rate of product synthesis, h^{-1}

K_S – substrate saturation constant, g L^{-1}
K^*_S – substrate saturation constant relevant for product formation, g L^{-1}
k_{sub} – specific rate of substrate consumption, h^{-1}
q_{ox} – specific rate of oxygen consumption, h^{-1}
q – volumetric substrate flow rate, L h^{-1}
V_c – reactor volume during continuous operation, L
V_L – initial reactor volume during fed batch operation, L

Greek

α – coefficient of inhibition of product synthesis, dimensionless
β_i – proportionality coefficient, g^{-1} L
γ_{DO} – concentration of dissolved oxygen, g L^{-1}
γ^*_{DO} – concentration of dissolved oxygen at saturation, g L^{-1}
γ_{DOSO} – concentration of dissolved oxygen in nutrient feed, g L^{-1}
γ_P – product concentration, g L^{-1}
γ_S – substrate concentration, g L^{-1}
γ_{SO} – substrate concentration in nutrient feed, g L^{-1}
γ_X – biomass concentration, g d. m. L^{-1}

Acronyms

BC – batch cultivation
CC – continuous cultivation
FBC – fed batch cultivation
MCA – renin enzyme activity (milk clotting activity)

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