Invited Paper

Modelling of a Batch Whey Cultivation of *Kluyveromyces marxianus var. lactis* MC 5 with Investigation of Mass Transfer Processes in the Bioreactor

Mitko Petrov[1*], Tatiana Ilkova[1], Juris Vanags[2]

[1]*Institute of Biophysics and Biomedical Engineering*
Bulgarian Academy of Sciences
Acad. George Bonchev Str., Bl. 105, 1113 Sofia, Bulgaria
E-mails: mpetrov@biomed.bas.bg, tanja@biomed.bas.bg

[2]*Join Stock Company "Biotehniskais Centrs"*
27 Dzerbenes Str., LV-1006 Riga, Latvia
E-mail: btc@edi.lv

[*]*Corresponding author*

Received:February 19, 2015 **Accepted: April 04, 2015**

Published: April 20, 2015

Abstract: *This study presents a mathematical model of a batch fermentation of lactose oxidation from a natural substratum in a cultivation by the strain Kluyweromyces marxianus var. lactis MC 5. In the model of the process, the mass transfer in the bioreactor for oxygen concentration in the gas phase (GP) and in the liquid phase (LP) is based on the dispersion model of the GP. In addition, perfect mixing in LP is included. Nine models were investigated for specific growth rate and specific oxygen consumptions rate: Monod, Mink, Tessier, Aiba, Andrews, Haldane, Luong, Edward and Han-Levenspiel. In regard to the parameter estimation, the worst observed error was used for all experiments as an objective function. This approach is a special case of multi objective parameter estimation problems allowing the parameter estimation problem to become a min-max problem. The results obtained (values of criteria, relative error and statistics λ) for the specific growth rate showed that the best fit to experimental data is achieved when applying the Mink model. In a combination a Mink, and Monod, Mink, Luong, Haldane, and Han-Levenspiel are used for specific oxygen consumptions rate. Based on the investigation, it was discovered that the best fit belonged to the models of Mink & Haldane, Mink & Luong and Mink & Han-Levenspiel. Therefore, these particular models are used for modeling the batch processes.*

Keywords: *Whey fermentation, Specific growth rate, Specific oxygen consumption rate, Kinetic models, Strain Kluyveromyces marxianus var. lactis.*

Introduction

The modelling of bioprocesses is a very important issue, which is determining for the discovery of radical principles for microbial synthesis. The dynamics of the biotechnological process is described by a mass balance equation because of the application of radical process parameters such as cell density, substrate concentration, profitable product, oxygen concentrations, temperature, pH and all once [20].

The cultivation of lactose oxidation from natural substratum in fermentation of *Kluyweromyces marxianus var. lactis* MC 5, using non-conventional ways for receiving

unicellular proteins, is not well studied. Therefore, a general mathematical model of the microbial synthesis does not exist because of the extreme complexity and great variety of living activity of microorganisms, although various models of the biotechnological process as well as of different parts of whey fermentation [1, 2] exist.

The bioreactors, where the energy is transferred simultaneously in the gas and liquid phase, appeared universal because of the availability of definite intensity of the mass-transfer and the mixing. The largest dispersion of the gas in the liquid is reached through mechanical mixing of the environment because of the well-built turbulence. When there is enough large gas hold-up, it creates a big relative surface of the phase contact and allows cultivation of cultural environment with components that have a large difference in density. This advantage of the apparatuses with mechanical mixing is the reason for their wide use. They are used mostly in the production of enzymes, amino acids, antibiotics, etc. Mass transfer processes in bioreactors exert immediate and essential influence on the growth and the development of cell population. The modelling of these processes is based on the equation of convective diffusion [4, 5, 14-16].

In this study, batch cultivations in the stirred tank of lactose oxidation from natural substratum in fermentation of the strain *Kluyweromyces Marxianus var. lactis MC 5* are investigated.

Materials and methods

Experimental investigations

Six fermentations have been carried out in aerobic batch and fed-batch cultivation of *Kluyweromyces marxianus var. lactis* MC5 strain. A laboratory bioreactor ABR 02M with a capacity of 2 L has been used. The strain has been cultivated under the following conditions [1, 2, 7-12]:

1. A nutrient medium with a whey ultra-filtrate with lactose concentration of 44 $g \cdot l^{-1}$ as a basic component is used. The ultra-filtrate is obtained from whey which was derived in the process of white cheese production and deproteinized by ultrafiltration on LAB 38 DDS with a membrane of the GR 61 PP type, under the following conditions:
   temperature T_1 = 40-43 °C
   input pressure P_{in} = 0.65 MPa
   output pressure P_{out} = 0.60 MPa
2. The ultra-filtrate is used in its native condition with lactose concentration of 44 $g \cdot l^{-1}$. The nutrient medium consists of:
   (NH)HPO 0.6%
   yeast autolisate 5.0%
   yeast extract 1.0%
   pH = 5.0-5.2
3. The velocity of the air flow is 60.0 $1 \cdot 1^{-1} \cdot h^{-1}$ up to the 4^{th} hour and 120.0 $1 \cdot 1^{-1} \cdot h^{-1}$ up to the end of the process under continuous mixing N = 800 rpm.
4. Temperature is T_2 = 29 °C.
5. Duration of the cultivation is t_f = 10 hours.

The following changes of the microbiological process (lactose conversion in yeasts cells to protein) have been studied during the strain growth:

a) the lactose concentration in the fermentation medium in oxidation and assimilation of lactose by *Kluyweromyces marxianus var. lactis* MC5 is determined by enzyme methods through UV tests (Boehringer Manheim, Germany, 1983);
b) the concentration of the cell mass and the protein contents is determined on the basis of the nitrogenous contents (Kjeltek system 1028);
c) the concentration of the dissolved oxygen in the fermentation medium in the process of oxidation and assimilation of lactose is determined by an oxygen sensor.

Kinetic models

The batch model of the processes includes the dependence on concentrations of the basic energetic substrates: oxygen concentration in the gas and liquid phase, cell mass concentration and lactose. The model of the mass transfer in the bioreactor considering oxygen concentration in the gas phase (GP) and the liquid phase (LP) is based on the dispersion model of the GP and perfect mixing in the LP. The change of the concentration in the gas phase (C_G) is described by a diffusion model at steady-state condition. The change of gas concentration in the liquid phase (C_L) is described by a perfectly mixed model. The dissolved oxygen concentration equilibrium (C^*) is determined as an average value of dissolved oxygen concentration in bioreactor liquid level. With these assumptions, the model of mass transfer is as follows [8, 10, 14-16]:

$$D_L \frac{d^2 C_G}{dz^2} - W_G \frac{dC_G}{dz} - \frac{k_l a}{\varepsilon_G}\left(\frac{C_G}{m_L} - C_L\right) = 0, \tag{1}$$

$$L\,C^* = \int_0^L C_G \; dz, \tag{2}$$

$$\frac{dC_L}{dt} = \frac{k_l a}{1-\varepsilon_G}\left(\frac{C^*}{m_L} - C_L\right) - \frac{1}{Y_{X/C}}\mu X. \tag{3}$$

The models of cell and substrate are described assuming that they are perfectly mixed in the bioreactor:

$$\begin{aligned} \frac{dX}{dt} &= \mu X \\ \frac{dS}{dt} &= -\frac{1}{Y_{X/S}}\mu X \end{aligned}, \tag{4}$$

where: C_G is the dissolved oxygen concentration in the gas phase, [kg·m^{-3}]; C_L – the dissolved oxygen concentration in the liquid phase, [kg·m^{-3}]; X – the biomass concentration, [kg·m^{-3}]; C^* – the dissolved oxygen concentration equilibrium, [kg·m^{-3}]; S – the substrate concentration, [kg·m^{-3}]; t – the process time, [h]; μ – the specific growth rate, [h^{-1}]; $Y_{X/S}$ and $Y_{X/C}$ – the yield coefficients, [kg·kg^{-1}]; $k_l a$ – the volumetric oxygen mass transfer coefficient, [h^{-1}]; ε_G – the gas phase hold-up, [%]; m_L – the Henry's law constant.

The initial and boundary conditions are given as follows:

$X(0) = 0.2$ kg·m^{-3}, $S(0) = 44$ kg·m^{-3}, $C_L(0) = 6.70 \cdot 10^{-3}$ kg·m^{-3}, and $C_G(0) = 0.21$ kg·m^{-3}.

$$z = 0 \rightarrow \quad W_G\, C_G^0 = W_G\, C_G - D_L \frac{dC_G}{dz}$$
$$z = L \rightarrow \quad \frac{dC_G}{dz} = 0$$

Including of dimensionless coordinate $\chi = z/L$, Eq. (1) and boundary conditions look like:

$$\frac{1}{Pe}\frac{d^2C_G}{d\chi^2} - \frac{dC_G}{d\chi} - a_1(C_G - m_L\, C_L) = 0, \tag{5}$$
$$\chi = 0: \quad C_G^0 = C_G - \frac{1}{Pe}\frac{dC_G}{d\chi}$$
$$\chi = 1: \quad \frac{dC_G}{d\chi} = 0$$
$$C^* = \int_0^1 C_G(\chi)\, d\chi, \tag{6}$$

where Pe is the *Peklet's* number, $\mathrm{Pe} = \frac{W_G L}{D_L}$, and $a_1 = \frac{k_l a\, L}{W_G\, \varepsilon_G\, m_L}$.

The model (1)-(4) can be vastly simplified if Eq. (1) is determined separately and its determination is included in the model (3), (4). The determination is considered in the type [15, 16]:

$$C_G(\chi, t) = A_0 \exp(r_1\chi) + B_0 \exp(r_2\chi) + C_0, \tag{7}$$

where: $A_0 = a_2 B_0; C_0 = C_G^0 - a_3 B_0$, $r_{1,2} = 0.5\mathrm{Pe} \pm \sqrt{0.25\mathrm{Pe}^2 + a_1\mathrm{Pe}}$,

$a_2 = -(r_2 / r_1)\, exp(r_2 - r_1)$, $\quad a_3 = 1 + a_2 - (a_2 r_1 + r_2)/\mathrm{Pe}$.

The dissolved oxygen concentration equilibrium is determined by the equation:

$$C^* = A_0[\exp(r_1) - 1]/r_1 + B_0[\exp(r_2) - 1]/r_2 + C_0. \tag{8}$$

The constant B_0 is determined from (8) by $t = 0$:

$$B_0 = \left(C_G^0 - m_L\, C_L^0\right)/a_4, \quad a_4 = a_3 + a_2[1 - \exp(r_1)]/r_1 + [1 - \exp(r_2)]/r_2.$$

The power input for the dispersion systems during the gas-liquid and the liquid phase following relations is determined by [8-10]:

$$P_G = 0.21\left(Q_G / N d^3\right)^{-0.1} P_L^{0.8}, \; P_L = 60.9\,\rho\, N^3 d^5\, \mathrm{Re}^{-0.4}. \tag{9}$$

Parameters ε_G and $k_l a$ can be determined by:

$$\varepsilon_G = 0.53\left(Q_G / N d^3\right)^{-0.014},\ k_l a = 52\left(P_G / V\right)^{0.38}\left(4 Q_G / \pi D^2\right)^{0.23}, \quad (10)$$

where D is the bioreactor diameter, [m]; d – impeller diameter, [m]; D_L – the dispersion coefficient, [$m^2 \cdot s^{-1}$]; N – the agitation speed, [s^{-1}]; P_G – the power input in the gas phase, [W]; P_L – the power input in the liquid phase, [W]; Q_G – the gas flow rate, [$m^3 \cdot s^{-1}$]; Re – the Reynold's number, $\mathrm{Re} = \rho N d^2 / \eta$; S – the substrate concentration, [$kg \cdot m^{-3}$]; V – the working volume, $V = 0.25 \pi D^2 L$, [m^3]; ρ – the liquid density, [$kg \cdot m^{-3}$]; η – the liquid dynamic viscosity, [Pa·s]; ρ_G – the aeration gas density, [$kg \cdot m^{-3}$].

The model structures for the specific rates are unknown, so nine models are tested in the study [4, 6, 13, 18, 19, 21]. Both classical specific rates and modified ones with regard to dissolved oxygen concentration are summarized in Table 1.

Table 1. Tested model structures

Model	$\mu(S)$	$\mu(C_L)$	Parameters
Monod	$\frac{\mu_m S}{K_S + S}$	$\frac{\mu_m C_L}{K_C + C_L}$	$\mu_m, K_S / \mu_m, K_C$
Mink	$\frac{\mu_m S^2}{K_S + S^2}$	$\frac{\mu_m C_L^{\,2}}{K_C + C_L^{\,2}}$	
Tessier	$\mu_m\left(1 - \exp^{(-S/K_S)}\right)$	$\mu_m\left(1 - \exp^{(-C_L/K_C)}\right)$	
Aiba	$\frac{\mu_m S}{K_S + S}\exp^{(-S/K_{SI})}$	$\frac{\mu_m C_L}{K_C + C_L}\exp^{(-C_L/K_{CI})}$	$\mu_m, K_S, K_{SI} /$ μ_m, K_C, K_{CI}
Andrews	$\frac{\mu_m S}{(K_S + S)(1 + S / K_{SI})}$	$\frac{\mu_m C_L}{(K_C + C_L)(1 + C_L / K_{CI})}$	
Haldane	$\frac{\mu_m S}{K_S + S + S^2 / K_{SI}}$	$\frac{\mu_m C_L}{K_C + C_L + C_L^{\,2} / K_{CI}}$	
Luong	$\frac{\mu_m S}{K_S + S} R_S^n$	$\frac{\mu_m C_L}{K_C + C_L} R_C^n$	$\mu_m, K_S, S_m /$ μ_m, K_C, C_m
Edward	$\frac{\mu_m S}{K_S + S + (S^2 / K_{SI})(1 + S / K)}$	$\frac{\mu_m C_L}{K_C + C_L + (C_L^{\,2} / K_{CI})(1 + C_L / K)}$	$\mu_m, K_S, K_{SI}, K /$ μ_m, K_C, K_{CI}, K
Han-Levenspiel	$\frac{\mu_m S R_S^n}{S + K_S R_S^m}$	$\frac{\mu_m C_L R_C^n}{C_L + K_C R_C^m}$	$\mu_m, K_S, S_m, n, m /$ μ_m, K_C, C_m, n, m

In the Table 1 $R_S = (1 - S / S_m)$; $R_C = (1 - C_L / C_m)$; μ_m is the maximum specific growth rate, [h^{-1}]; K_S, K_C – the saturation constants, [$kg \cdot m^{-3}$]; K_{SI}, K_{CI} – the inhibition constants in different models, [$kg \cdot m^{-3}$]; K – the constant in the *Edward* model; S_m, C_m – the critical inhibitor concentration, above which the reactions stop, [$kg \cdot m^{-3}$]; m – the constant in the *Han-Levenspiel* model; n – the constant in the *Luong* and the *Han-Levenspiel* models.

Evaluation of the model parameters

The mathematical estimation of the model parameters is based on the minimization of some quantities that can be calculated and the estimation of a function of parameters. If the model under consideration is linear, the estimation is generally an easy task. However, there is no general theory for nonlinear parameter estimations. The least-squares error is commonly employed as a criterion to inspect how close the computed profiles of the state variables come to the experimental observations. In this study, we have considered the time weighted least-squares error as a criterion for each experiment. The criterion is expressed in the form [21]:

$$Q_k = \frac{1}{N_{\exp}} \sum_{j=1}^{N_{\exp}} t_j \left(\frac{(X_e(t_j) - X_m(t_j))^2}{X^2_{e\max}(t_j)} + \frac{(S_e(t_j) - S_m(t_j))^2}{S^2_{e\max}(t_j)} + \frac{(C^e_L(t_j) - C^m_L(t_j))^2}{C^2_{Le\max}(t_j)} \right), \quad (11)$$

where N_{exp} is the number of experiments, and t_j – time partitions.

The least-squares regression sums up every observed error in (11) to the yield of an objective function. For the parameters estimation, we have considered the worst observed error for all experiments as an objective function. This approach is a special case of multiobjective parameter estimation problems so that the parameter estimation problem becomes a min-max problem [21]:

$$\min_{\mathbf{u}} Q = \min_{\mathbf{u}} \max_{k} \{ Q_k, k = 1, ..., N_{\exp} \}, \quad (12)$$

where **u** is a vector of the estimated parameters.

Now, the min-max problem can be solved by the subroutine BCPOL from the IMSL library of COMPAQ Visual FORTRAN 90 [3]. The routine BCPOL uses the complex method to find a minimum point of a function of n variables. The method is based on function comparison; no smoothness is assumed. All computations have been performed on Dual Core AMD Athlon II 2900 MHz computer using Microsoft Windows XP Pro Edition operating system.

Models validation

The best dependences are defined by the statistical criteria: experimental correlation coefficient (R^2_E), experimental Fisher function (F_E), relative error (S_L) and statistics λ for the different mixing systems and the models of the specific growth rate.

In that statistics λ has $F(M, N_{\exp} - M)$ distribution. Statistics λ is defined with [4]:

$$\lambda = \frac{(N_{\exp} - M)}{(N_{\exp} - 1)} \frac{N_{\exp}}{M} \sum_{j=1}^{M} \frac{\Delta^2_j}{S_j}, \quad (13)$$

where: $\Delta^2_j = \frac{1}{N_{\exp}} \sum_{i=1}^{N_{\exp}} \left(X_e(t_i) - X_m(t_i)\right)^2 + \left(S_e(t_i) - S_m(t_i)\right)^2 + \left(C^e_L(t_i) - C^m_L(t_i)\right)^2$,

$S_j = \frac{1}{N_{\exp} - 1} \sum_{i=1}^{N_{\exp}} \left(-\Delta_{i,j}\right)^2 \Delta_j$, M – number of kinetics variables.

The relative error S_L is determined with the help of the following equation [4]:

$$S_L = \sqrt{\frac{1}{(N_{\exp} - M)} \sum_{j=1}^{N_{\exp}} \frac{\left(X_e(t_j) - X_m(t_j)\right)^2}{X_e^2(t_j)} + \frac{\left(S_e(t_j) - S_m(t_j)\right)^2}{S_e^2(t_j)} + \frac{\left(C_{Le}(t_j) - C_{Lm}t_j\right)^2}{C_{Le}^2(t_j)}} . \quad (14)$$

Results and discussion

Identification procedures of differential equations system (3)-(4) start with application of classical model structures of nine specific rates $\mu(S)$, as presented in the second column of Table 1. Further the same investigation is repeated using the modified specific rates $\mu(C_L)$, listed in the third column of Table 1.

Considering system (3)-(4) the basic indexes of mass transfer and mixing of the process have the following values:

$(P_G/V) = 0.49$ W·m^{-3}; $m_L = 36.14$; for
$Q_G = 60$ m^3·m^{-3}·h^{-1} $\rightarrow$ $\varepsilon_G = 23.05\%$, $k_la = 173.2$ h^{-1}, and
$Q_G = 120$ m^3·m^{-3}·h^{-1} $\rightarrow$ $\varepsilon_G = 26.34\%$, $k_la = 202.7$ h^{-1}.

The obtained statistical results (criterion value Q, statistics λ and relative errors S_L) are shown in Table 2, respectively for classical and modified specific rate structures.

Table 2. Statistical results for all investigated model structures

Model	in case of $\mu(S)$					in case of $\mu(C_L)$				
	$Q\cdot10^{-3}$	λ	$S_L(X)$	$S_L(S)$	$S_L(C_L)$	$Q\cdot10^{-3}$	λ	$S_L(X)$	$S_L(S)$	$S_L(C_L)$
Monod	201.4	0.230	0.431	0.570	8.369	**9.7**	**536.649**	**0.220**	**0.273**	**0.276**
Mink	**55.0**	**19.785**	**0.244**	**0.565**	**3.466**	**9.6**	**130.203**	**0.255**	**0.375**	**0.280**
Teisser	319.3	0.128	0.853	0.889	8.451	11.2	94.428	0.263	1.102	0.285
Aiba	148.7	0.350	0.410	0.560	7.071	14.8	61.457	0.216	0.657	0.317
Andrews	75.8	1.950	0.320	0.409	5.672	11.0	74.998	0.223	0.720	0.267
Haldane	78.5	1.106	0.289	0.659	2.482	**15.0**	**60.659**	**0.212**	**0.655**	**0.347**
Luong	89.4	0.777	0.305	0.666	3.212	**9.8**	**278.221**	**0.214**	**0.267**	**0.266**
Edward	94.1	0.738	0.303	0.662	3.543	15.0	64.284	0.211	0.655	0.343
Han-Levenspiel	83.0	0.945	0.293	0.660	2.909	**10.9**	**208.868**	**0.220**	**0.330**	**0.297**

The tabular coefficient of Fisher is $F_T(8,3) = 3.42$ and for statistics λ – $F_T'(3,8) = 4.13$. The tabular correlation coefficient is $R_T^2(8) = 0.632$ [17]. The experimental values of the correlation coefficients are not presented in the table because all coefficients have values $R_E^2 > 0.90$, and the experimental Fisher coefficient (F_E) has an order of one. This shows that all models are adequate according to mentioned above criteria, except the statistics λ.

The statistical indexes in Table 2 show that only the model of *Mink* has the best fit in case of $\mu(S)$. For the rest of the models the statistics λ is smaller from the theoretical value $F_T'(3,8) = 4.13$, which means that they do not result in a good fit with the experimental data.

The models of *Monod*, *Mink*, *Luong* and *Han-Levenspiel* have the best fit in case of $\mu(C_L)$ (Table 2). In spite of higher indexes, the *Haldane* model is also included in the group of the structures with the best fit, because it was successfully used for the modelling of the same process in previous authors' investigations [7, 9, 17]. Thus the following five combinations

between the model structures showed best fit in case of $\mu(S)$ and $\mu(C_L)$ are proposed for further identification procedures:

1) Model of *Mink* as $\mu(S)$ and *Haldane* as $\mu(C_L)$

$$\mu(S, C_L) = \frac{\mu_m S^2}{\left(K_S + S^2\right)} \frac{C_L}{\left(K_C + C_L + C_L^2 / K_I\right)} \tag{15}$$

2) Model of *Mink* as $\mu(S)$ and *Monod* as $\mu(C_L)$

$$\mu(S, C_L) = \frac{\mu_m S^2}{\left(K_S + S^2\right)} \frac{C_L}{\left(K_C + C_L\right)} \tag{16}$$

3) Model of *Mink* as $\mu(S)$ and *Mink* as $\mu(C_L)$

$$\mu(S, C_L) = \frac{\mu_m S^2}{\left(K_S + S^2\right)} \frac{C_L^2}{\left(K_C + C_L^2\right)} \tag{17}$$

4) Model of *Mink* as $\mu(S)$ and *Luong* as $\mu(C_L)$

$$\mu(S, C_L) = \frac{\mu_m S^2}{\left(K_S + S^2\right)} \frac{C_L}{\left(K_C + C_L\right)} \left(1 - \frac{C_L}{C_m}\right)^n \tag{18}$$

5) Model of *Mink* as $\mu(S)$ and *Han-Levenspiel* as $\mu(C_L)$

$$\mu(S, C_L) = \frac{\mu_m S^2}{\left(K_S + S^2\right)} \frac{C_L}{\left(C_L + K_C (1 - C_L / C_m)^m\right)} \left(1 - \frac{C_L}{C_m}\right)^n \tag{19}$$

The statistical indexes of the investigated model combinations (models (15)-(19)) are shown in Table 3.

Table 3. Statistical results for models (15)-(19)

Models for $\mu(S, C_L)$	$Q \cdot 10^{-3}$	λ	$S_L(X)$	$S_L(S)$	$S_L(C_L)$
Mink & Haldane	**9.158**	**825.030**	**0.212**	**0.281**	**0.272**
Mink & Monod	8.971	205.722	0.228	0.267	0.223
Mink & Mink	9.387	413.005	0.222	0.276	0.335
Mink & Luong	**9.099**	**563.251**	**0.217**	**0.280**	**0.198**
Mink & Han-Levenspiel	**8.977**	**531.835**	**0.222**	**0.259**	**0.256**

Table 3 shows that all investigated combinations of the models have a very good fit. The models *Mink & Haldane* (Eq. (15)), *Mink & Luong* (Eq. (18)), and *Mink & Han-Levenspiel* (Eq. (19)), have the best fit.

The estimated parameters of the investigated models are shown in Table 4.

The results after simulations for the concentrations of biomass (X), substrate (S) and oxygen (C_L) for the batch cultivation of *Kluyweromyces Marxianus var. lactis* MC5 with all models (15)-(19) are shown in Figs. 1-3, respectively.

Table 4. Parameters of models (15)-(19)

Models	μ_m	K_S	K_C	$Y_{X/S}$	$Y_{X/C}$	K_{CI}	C_m	n	m
Mink & Haldane	0.866	3.469	0.606	0.396	0.164	17.846	–	–	–
Mink & Monod	0.693	0.423	0.488	0.398	0.152	–	–	–	–
Mink & Mink	0.622	0.157	0.143	0.395	0.161	–	–	–	–
Mink & Luong	0.736	0.733	0.491	0.399	0.158	–	24.335	0.313	–
Mink & Han-Levenspiel	0.695	5.007	0.410	0.395	0.160	–	21.872	0.183	1.156

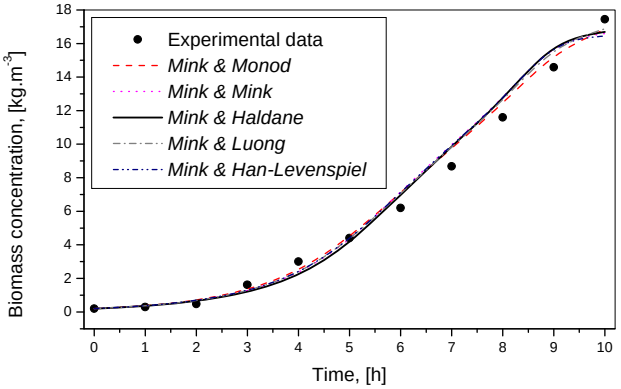

Fig. 1 Experimental and simulation results for biomass concentration

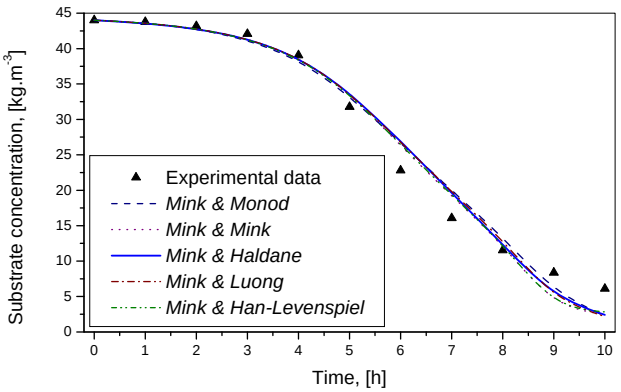

Fig. 2 Experimental and simulation results for substrate concentration

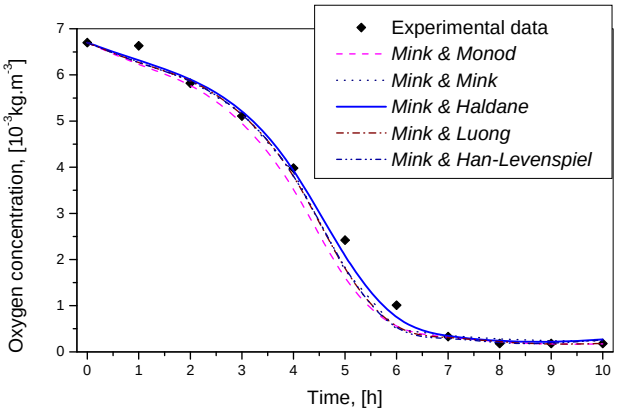

Fig. 3 Experimental and simulation results for oxygen concentration

The obtained results for the optimisation criterion Q, relative errors S_L and statistics λ (Table 4, Figs. 1-3) show that all models can be used for the modelling of a batch cultivation of *Kluyweromyces marxianus var. lactis* MC 5.

Conclusions

This study evaluates a mathematical model of a batch cultivation of lactose oxidation from natural substratum in the fermentation of the strain *Kluyweromyces marxianus var. lactis* MC 5. In the model the mass transfer in the bioreactor for the oxygen concentration in the *GP* and *LP* is based on the dispersion model of *GP* and perfect mixing in *LP*. Eighteen model structures of specific rates in respect to S and C_L have been investigated, namely *Monod*, *Mink*, *Tessier*, *Aiba*, *Andrews*, *Haldane*, *Luong*, *Edward* and *Han-Levenspiel*. The obtained results (criteria value, correlation and Fisher coefficients, relative error and statistics λ) in case of $\mu(S)$ show that the model of *Mink* has the best fit. Other models have low values of the evaluation criteria. In case of $\mu(C_L)$ all investigated models are adequate and can be used for modelling.

Based on obtained results the following model variations have been proposed: *Mink* as $\mu(S)$ in a combination respectively with models of *Haldane*, *Monod*, *Mink*, *Luong*, and *Han-Levenspiel* as $\mu(C_L)$. After performed identification procedures the best statistical indicators are shown by models *Mink* & *Haldane*, *Mink* & *Luong*, and *Mink* & *Han-Levenspiel*.

Considered model structures of specific rates are here applied in case of a batch mode of the fermentation process. Further models investigations might be performed when modelling fed-batch mode of the fermentation process.

The elaborated algorithms and programs on Compaq Visual FORTRAN 90 might be used for modelling of other fermentation processes too.

References

1. Angelov P., D. Filev, N. Kasabov (Eds.) (2010). Evolving Intelligent Systems: Methodology and Applications, IEEE Press Series on Computational Intelligence, Hardcover, John Wiley & Sons.
2. Angelov P., E. Simova, D. Beshkova, G. Frengova (1996). Control of Cell Protein Synthesis from *Kluyveromyces marxianus var. lactis* MC5, Biotechnology and Biotechnological Equipment, 10, 44-50.
3. COMPAQ Visual FORTRAN Programmer's Guide, v. 6.6, Compaq Computer Corporation, Houston, Texas, 2001.
4. Giridhar R., A. Srivastava (2000). Model Based Constant Feed Fed-batch *L-sorbose* Production Process for Improvement in *L-sorbos*e Productivity, Chemical and Biochemical Engineering Quarterly, 14(4), 133-140.
5. Kafarov V., A. Vinarov, L. Gordeev (1985). Modelling and Systematic Analysis of Biochemical Production, Moscow, Lesnaya promishlenost (in Russian).
6. Kim D.-J., J.-W. Choi, N.-C. Choi, B. Mahendran, C.-E. Lee (2005). Modeling of Growth Kinetics for *Pseudomonas spp.* during Benzene Degradation, Appl Microbiol Biotechnol, 69, 456-462.
7. Petrov M. (2006). A Multiple-objective Optimization of Whey Fermentation in Stirred Tank, Int J Bioautomation, 5, 39-48.

8. Petrov M., St. Tzonkov (1999). Modelling of Mass Transfer and Optimization of Stirred Bioreactors, Bioprocess Engineering, 21(1), 61-63.
9. Petrov M., St. Tzonkov (2009). Multi-objective Optimization of the Mass-transfer in Stirred Tank Bioreactors, Int J Bioautomation, 13(4), 173-184.
10. Petrov M., T. Ilkova T., St. Tzonkov., U. Viesturs (2004). Modeling, Optimization and Optimal Control of Small Scale Stirred Tank Bioreactors, Int J Bioautomation, 1, 68-83.
11. Petrov M., T. Ilkova, St. Tzonkov (2005). Modelling and Fuzzy Optimization of Whey Fermentation by *Kluyveromyces marxianus var. lactis* MC 5, Chemical and Biochemical Engineering Quarterly, 19(1), 49-55.
12. Petrov M. (2008). Multiple Objective Optimization and Optimal Control of Fermentation Processes, Int J Bioautomation, 10, 21-30.
13. Saravanan P., K. Pakshirajan, P. Saha (2011). Kinetics of Phenol Degradation and Growth of Predominant Pseudomonas Species in a Simple Batch Stirred Tank Reactor, Bulgarian Chemical Communications, 43(4), 502-509.
14. Schmalzriedt S., M. Jenne, M. Reuss (1995). Modeling of Stirred Tank Bioreactors, Proc. of 6th International Conference on Computer Application in Biotechnology, Garmish-Partenkirchen, Germany, 159-164.
15. Stenberg O., B. Andersson (1988). Gas-liquid Mass Transfer in Agitated Vessels. I. Evaluation of the Gas-liquid Mass Transfer Coefficient from Transient-response Measurement, Chem Eng Science, 43(3), 719-724.
16. Stenberg O., B. Andersson (1988). Gas-Liquid Mass Transfer in Agitated Vessels. II. Modeling of Gas-Liquid Mass Transfer, Chem Eng Science, 43(3), 725-730.
17. Stoyanov S. (1983). Optimisation of Technological Objects, Technika, Sofia (in Bulgarian).
18. Sudipta D., S. Mukherjee (2010). Performance and Kinetic Evaluation of Phenol Biodegradation by Mixed Microbial Culture in a Batch Reactor, Int J of Water Resources and Environmental Engineering, 2(3), 40-49.
19. Thĕodore K., T. Panda (1999). Effect of Glucose Level on the Batch Production of b-1,3-Glucanase by *Trichoderma harzianum* and Cell Growth, Bioprocess Engineering, 20, 309-311.
20. Tzonkov St. (Ed.) (2010). Contemporary Approaches to Modelling, Optimisation and Control of Biotechnological Processes, Prof. Marin Drinov Academic Publishing House, Sofia.
21. Wang F.-S., S. Tzu-Liang, J. Horng-Jhy (2001). Hybrid Differential Evolution for Problems of Kinetic Parameter Estimation and Dynamic Optimization of an Ethanol Fermentation Process, Ind & Eng Chem Res, 40, 2876-2885.

Assoc. Prof. Mitko Petrov, Ph.D.
E-mail: mpetrov@biomed.bas.bg

Mitko Petrov (born 1959) graduated from the Technical University – Sofia in 1987 as a mechanical engineer. He has worked as an Associate Professor at the Institute of Biophysics and Biomedical Engineering – Bulgarian Academy of Sciences since 1988. He achieved his Ph.D. degree in 2004. He has been an Associate Professor since 2007. His scientific interests are in the fields of bioprocess systems, modelling and optimization of bioprocesses, modelling and optimization of apparatus of bioprocess systems, and modelling of ecological systems. He has about 150 scientific publications with more than 20 known citations.

Assoc. Prof. Tatiana Ilkova, Ph.D.
E-mail: tanja@biomed.bas.bg

Tatiana Ilkova was born in 1970. She received the M. Sc. Degree in Engineering of Biotechnology (1995) and Ph.D. Degree (2008) from the Technical University – Sofia. At present, she is an Associate Professor at the Institute of Biophysics and Biomedical Engineering – Bulgarian Academy of Sciences. Her scientific interests are in the fields of bioprocess systems, modelling and optimization of bioprocesses and modelling of ecological systems. She has about 140 scientific publications with more than 50 known citations.

Res. Assoc. Juris Vanags, Dr.Sc.Eng.
E-mail: btc@edi.lv

Juris Vanags (born 1954) graduated from the University of Latvia in 1983 as a physical engineer. He worked at the Institute of Microbiology, LAS as a researcher (1984-1990). Since 1990, he has worked as a researcher at the Latvian State Institute of Wood Chemistry (Laboratory of Bioengineering) Since 1996, he has also worked as Chairman of the Board at Join Stock Company "Biotehniskais Centrs". He received his Dr. Sc. Eng. degree in 1993. His scientific interests are process automation, bioreactor design and bioprocess control. He has about 60 scientific publications and 2 patents.