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Modelling and multicriteria analysis for selection of growth rate models for batch cultivation of *Kluyveromyces marxianus var. lactis* MC 5 yeast. Part I: Modelling with different types of growth rate models

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To explain the kinetics of cell growth, this work examined ten unstructured models for growth rate – *Monod*, *Mink*, *Tessier*, *Moser*, *Aiba*, *Andrews*, *Haldane*, *Luong*, *Edward* and *Han-Levenspiel* in batch cultivation of *Kluyweromyces marxianus var. lactis* MC 5 yeast. For the first time, the process of modelling was done using two separate models for growth kinetics separately for the two main substrates – lactose and oxygen. The following criteria were used to validate the models: sum of squared weighted residuals, statistics λ, relative error, Fisher's experimental function, and experimental correlation coefficient. Each of the criteria was used separately for biomass / lactose or biomass / oxygen. At the end of the study, the criteria of the growth rate models were compared depending on lactose-only or oxygen-only. The model with the most criteria that meet the minimum and maximum requirements for lactose is the *Han-Levenspiel* model. In terms of oxygen, the *Moser* model has the best results.

Keywords: Modelling, Growth rate models, *Kluyveromyces marxianus var. lactis* MC 5 yeast, statistical criteria

INTRODUCTION

Strains belonging to the yeast species *Kluyveromyces marxianus* have been isolated from a wide variety of habitats. For this reason, this strain has been used for various biotechnological applications: production of enzymes, unicellular protein, aromatic compounds and ethanol, reduction of lactose content in food products, production of bioelements from cheese-whey, etc. [1].

There is currently no general mathematical model of yeast *Kluyveromyces marxianus var. lactis* MC 5 due to the extreme complexity and the great variety of vital activities of yeast and the great variety of strains. Although, there are different models of the biotechnological process and of different parts of whey fermentation [2].

The aim of this work is to model the *Kluyweromyces marxianus var. lactis* MC 5 yeast in batch cultivation with ten different models for growth rate, separately for the two main substrates – lactose and oxygen and to choose the most suitable models.

MATERIALS AND METHODS

Experimental investigation

The *Kluyveromyces marxianus var. lactis* MC 5 yeast was cultivated under the following conditions [3]:

1. Nutrient medium with basic component – whey ultrafiltrate with lactose concentration 44 g/L. The ultrafiltrate was derived from whey separated in production of white cheese and deproteinisation by ultrafiltration on LAB 38 DDS with membrane of the type GR 61 PP under the following conditions:
   - Temperature, t 40 – 43 °C;
   - Input pressure, P_{in} 0.65 MPa;
   - Output pressure, P_{out} 0.60 MPa.

   The ultrafiltrate was used in native condition with lactose concentration of 44 g/L. Nutrient medium consisted of:
   - $(NH)_4HPO$ 0.6%;
   - Yeast autolisate 5%;
   - Yeast extract 1%;
   - pH 5.0 – 5.2.

   The gas flow rate was Q_G = 60 L/L/h up to the 4th hour and Q_G = 120 L/L/h up to the end of the process under continuous mixing with n = 800 min^{-1}. The temperature was 29°C.
2. The changes of the microbiological process (lactose conversion in yeast's cells to protein) were studied during the strain growth:
   - lactose concentration in the fermentation medium in oxidation and assimilation of lactose by *Kluyweromyces Marxianus var. lactis* MC 5 was determined by enzyme methods and UV tests (Boehringer Mannheim, Germany, 1983);

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– concentrations of cell mass and protein contents were determined on the basis of the nitrogen content (Kjeltek system 1028);

– concentration of dissolved oxygen in the fermentation medium in the process of oxidation and assimilation of lactose was determined by an oxygen sensor. The oxygen sensor produced by LKB firm was used for the measurement of the oxygen concentration in the fermentation medium.

3. Duration of the cultivation was t_f = 12 hours. Six fermentations were carried out in aerobic batch cultivation of *Kluyweromyces Marxianus var. lactis* MC 5. The experimental investigations were carried out on a computer controlled laboratory bioreactor 2L-M with magnetic coupling.

In modelling the process, we used the mean values of the kinetic variables from the six fermentations.

Kinetic models

The model includes cell concentration in the dependence between concentrations of the basic energetic substrates – lactose and oxygen. In all our previous works [4-7] for modelling, optimisation and optimal control for the specific growth rate we have always used *Mink* model for lactose and *Haldane* model for oxygen:

$$\mu(S,C) = \frac{\mu_m S^2}{K_S + S^2} \frac{C}{K_C + C + C^2/K_{CI}}$$

where: S – lactose concentration, g/L; C – oxygen concentration, g/L; μ_m – maximal growth rate, h^{-1}; K_S, K_C and K_{CI} – kinetic parameters, g/g.

In contrast to the previously used approaches for process modelling, in this work, the kinetic models were developed separately for the two main substrates:

– *Biomass production from lactose-only*

$$\begin{aligned} \frac{dX}{dt} &= \mu(S)X \\ \frac{dS}{dt} &= -\frac{1}{Y_{X/S}}\frac{dX}{dt} \end{aligned} \quad (1)$$

where: t – process time, h; X – biomass concentration, g/L; $\mu(S)$ – growth rate of biomass from lactose, h^{-1}; $Y_{X/S}$ – yield coefficients of formation of the biomass from lactose, g/g.

– *Biomass production from oxygen-only*

$$\begin{aligned} \frac{dX}{dt} &= \mu(C)X \\ \frac{dC}{dt} &= -\frac{1}{Y_{X/C}}\frac{dX}{dt} + k_l a(C^* - C) \end{aligned} \quad (2)$$

where: $\mu(C)$ – growth rate of biomass from oxygen, h^{-1}; $Y_{X/C}$ – yield coefficients of formation of the biomass from oxygen, g/g; $k_l a$ – mass transfer coefficient, h^{-1}; C^* – maximal oxygen concentration in liquid phase, g/L.

The initial conditions of both models (Eqns. 1 and 2) were:

$X(0)$ = 0.2 g/L, $S(0)$ = 44 g/L; $C(0) = 6.65 \cdot 10^{-3}$ g/L, and $C^* = C(0)$.

System constraints of both models

Nearly all engineering processes have physical constraints. The lactose and oxygen concentrations must be positive all the time for the processes, otherwise, an unrealistic solution in the identification problem will be obtained [8]:

$$g_1(t,\mathbf{x}) = -S(t) \le 0 \quad (3)$$

$$g_3(t,\mathbf{x}) = -C(t) \le 0 \quad (4)$$

In addition, here are the following constraints for stochiometry by the processes:

$$g_2(t,\mathbf{x}) = \frac{S(0) - S(t)}{X(t) - X(0)} - \frac{1}{Y_{X/S}} \le 0 \quad (5)$$

$$g_4(t,\mathbf{x}) = \frac{k_l a(C^* - C)}{X(t) - X(0)} - \frac{1}{Y_{X/C}} \le 0 \quad (6)$$

where: x – vector of the kinetic parameters, $\mathbf{x} = \mathbf{x}[\mu_m^S, ..., Y_{X/S}]^{\mathrm{T}}$ for model (Eqns. 1), and $\mathbf{x} = \mathbf{x}[\mu_m^C, ..., Y_{X/C}, k_l a]^{\mathrm{T}}$ for model (Eqns. 2).

Specific growth rate models

The paper compares ten unstructured models [9-13]: M_1 – *Monod*, M_2 – *Mink*, M_3 – *Tessier*, M_4 – *Moser*, M_5 – *Aiba*, M_6 – *Andrews*, M_7 – *Haldane*, M_8 – *Luong*, M_9 – *Edward*, and M_{10} – *Han-Levenspiel* to explain the kinetics of cell growth (Table 1).

In Table 1: $\mathbf{Z} = [S, C]^{\mathrm{T}}$ – vector with basic energetic substrates lactose and oxygen, respectively; $\mu_m^{\mathbf{Z}}$ – maximum growth rate for lactose and oxygen, respectively, h^{-1}; $K_{\mathbf{Z}}$ – *Monod* saturation constants for cell growth on lactose and oxygen, g/L; α_M – *Moser* constant; $K_{\mathbf{Z}I}$ – inhibition constants for cell growth on lactose and oxygen, g/L; K – constant in *Edward* model, g/L; $\mathbf{Z}_m$ – critical inhibitor concentrations, above which the reaction stops, g/L; m, n – constants in the *Luong* and the *Han-Levenspiel* models.

Table 1. Tested growth rate models dependent on lactose or oxygen

Model	$\mu(\mathbf{Z})$
$M_1(\mathbf{Z})$	$\mu(\mathbf{Z}) = \frac{\mu_m^{\mathbf{Z}}\,\mathbf{Z}}{K_{\mathbf{Z}} + \mathbf{Z}}$
$M_2(\mathbf{Z})$	$\mu(\mathbf{Z}) = \frac{\mu_m^{\mathbf{Z}}\,\mathbf{Z}^2}{K_{\mathbf{Z}} + \mathbf{Z}^2}$
$M_3(\mathbf{Z})$	$\mu(\mathbf{Z}) = \mu_m^{\mathbf{Z}}\left(1 - \exp\left(-\mathbf{Z} / K_{\mathbf{Z}I}\right)\right)$
$M_4(\mathbf{Z})$	$\mu(\mathbf{Z}) = \frac{\mu_m^{\mathbf{Z}}\,\mathbf{Z}^{\alpha_M}}{K_{\mathbf{Z}} + \mathbf{Z}^{\alpha_M}},\ \alpha_M > 0$
$M_5(\mathbf{Z})$	$\mu(\mathbf{Z}) = \frac{\mu_m^{\mathbf{Z}}\,\mathbf{Z}}{K_{\mathbf{Z}} + \mathbf{Z}} \exp\left(-\mathbf{Z} / K_{\mathbf{Z}I}\right)$
$M_6(\mathbf{Z})$	$\mu(\mathbf{Z}) = \frac{\mu_m^{\mathbf{Z}}\,\mathbf{Z}}{(K_{\mathbf{Z}} + \mathbf{Z})\left(1 + \mathbf{Z} / K_{\mathbf{Z}I}\right)}$
$M_7(\mathbf{Z})$	$\mu(\mathbf{Z}) = \frac{\mu_m^{\mathbf{Z}}\,\mathbf{Z}}{K_{\mathbf{Z}} + \mathbf{Z} + \mathbf{Z}^2 / K_{\mathbf{Z}I}}$
$M_8(\mathbf{Z})$	$\mu(\mathbf{Z}) = \frac{\mu_m^{\mathbf{Z}}\,\mathbf{Z}}{K_{\mathbf{Z}} + \mathbf{Z}}\left(1 - \mathbf{Z} / \mathbf{Z}_m\right)^n$
$M_9(\mathbf{Z})$	$\mu(\mathbf{Z}) = \frac{\mu_m^{\mathbf{Z}}\,\mathbf{Z}}{K_{\mathbf{Z}} + \mathbf{Z} + \left(1 + \mathbf{Z} / K\right)\left(S^2 / K_{\mathbf{Z}I}\right)}$
$M_{10}(\mathbf{Z})$	$\mu(\mathbf{Z}) = \frac{\mu_m^{\mathbf{Z}}\,\mathbf{Z}}{\mathbf{Z} + K_{\mathbf{Z}}} \frac{\left(1 - \mathbf{Z} / \mathbf{Z}_m\right)^n}{\left(1 - \mathbf{Z} / \mathbf{Z}_m\right)^m}$

Criteria for evaluation of the model parameters

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 [14]:

$$\mathbf{Q} = \frac{1}{N}\sum_{i=1}^{N}\left(\frac{(X_e(t_i) - X_m(t_i))^2}{X_{e\max}^2} + \frac{(\mathbf{Z}_e(t_i) - \mathbf{Z}_m(t_i))^2}{\mathbf{Z}_{e\max}^2}\right) \quad (7)$$

where: $\mathbf{Q} = [Q_1, Q_2]^{\mathrm{T}}$, Q_1 and Q_2 – sums of the squares of weighted residuals lactose-only or oxygen-only; N – number of the experiments; t_i – time partitions; $X_e(t_i), X_m(t_i)$ – experimental and simulated values of biomass concentration, g/L; $X_{e\max}^2$ – maximal experimental value of biomass concentration in the end of the process, g/L; $\mathbf{Z}_e(t_i) = [S_e(t_i), C_e(t_i)]^{\mathrm{T}}$, $\mathbf{Z}_m(t_i) = [S_m(t_i), C_m(t_i)]^{\mathrm{T}}$ – experimental and simulated values of lactose and oxygen, respectively, g/L.

The constraints were included in the parameter estimation problem (Eqn. 7), in order to avoid unrealistic predicted values:

$$\min_{\mathbf{x}} \mathbf{J} = \mathbf{Q} + \sum_{i=1}^{4} w_i \int_{t=0}^{t_f} g_i(t, \mathbf{x})\, dt \quad (8)$$

where $\mathbf{x}$ is a vector of the estimated parameters; $\mathbf{J} = [J_1, J_2]^{\mathrm{T}}$, J_1 and J_2 – criteria of minimization for lactose-only and for oxygen-only; t_f – final time, h; $g_i(t, \mathbf{x})$ – system constraints, i = 1,…,4; w_i – weight of each constraint, $w_i = 10^2$. Such a large weight of each constraint was chosen to make it easier to identify models. If the constraints are satisfied, then $g_i(t, \mathbf{x}) = 0$.

Criteria for model validation

The best values are defined by the criteria:

– $C_1(S) \equiv J_1 \times 10^{-3}$ or $C_1(C) \equiv J_2 \times 10^{-3}$ – criteria for evaluation of parameters (Eqn. 8);

– $C_2(S)$ or $C_2(C)$ – statistic λ. The criteria C_2 was compared to the tabular Fisher coefficient $F_T^{\lambda}(\nu_1, \nu_2)$, where ν_1 – degree of freedom of model, and ν_2 – degree of freedom of experimental data, $\nu_2 = N - 2$;

– [$C_3(X)$ and $C_4(S)$] or [$C_3(X)$ and C_4(C)] – relative error (S_L) for the kinetic variables (X, S) or (X, C);

– [$C_5(X)$ and $C_6(S)$] or [$C_5(X)$ and C_6(C)] – experimental Fisher coefficient for the kinetic variables (X, S) or (X, C). The obtained values of criteria C_5 and C_6 were compared to the tabular values for Fisher coefficient (F_T) for degrees of freedom $F_T(\nu_2, \nu_1)$;

– [$C_7(X)$ and $C_8(S)$] or [$C_7(X)$ and $C_8(C)$] – experimental correlation coefficient R^2 for the kinetic variables (X, S) or (X, C). The obtained values of criteria C_7 and C_8 were compared to the tabular values of correlation coefficient ($R_T^2(\nu_2)$) with a degree of freedom ν_2.

Statistic λ, relative error S_L, Fisher coefficient (F) and correlation coefficient (R^2) are fully presented in [15].

RESULTS AND DISCUSSION

Modelling of the process from lactose-only or oxygen-only

In the first part of this work, the aim was to model the process separately (of course, the separation is artificial) for lactose and oxygen with 10 different growth rate models (Table 1). Here we were interested only in the results obtained for the criteria (C_1 – C_8) by which the studied models were evaluated.

An algorithm and a program of COMPAQ Visual FORTRAN 90 [16] were developed in order to identify the parameters in models (Eqns. 1 – 8). All computations were performed using HexaCore

AMD Phenom II X6 1075T, 3 GHz, 8 GB RAM, Windows XP operating system (32 bit).

Tables 2 and 3 show the results for the criteria by which the models (M_1 – M_{10}) were validated lactose-only (Eqns. 1), and oxygen-only (Eqns. 2).

Here it is time to specify that for criteria C_1 – C_6 we are looking for a minimum value, and for criteria C_7 and C_8 – for a maximum one. The best values of all criteria are marked in green. The maximum values for criteria C_1 – C_6, and the minimum values for criteria C_7 and C_8 are marked in red.

The minimal ($C_{i\min}$) and maximal ($C_{i\max}$) values of every criterion for lactose-only and oxygen-only are shown in Table 4.

From the presented results (Table 4) it can be seen that we have better values of the criteria in the modelling of the process depending on oxygen-only. Criterion C_1 for oxygen-only has better minimum and maximum values than that for lactose-only. The difference in the minimum values for criterion C_2 is minimal (only 3% in favour of criterion C_2 for lactose-only). Only for criterion $C_4(S)$ in lactose-only we have a better result. This should show us that the growth kinetics of biomass depend more on oxygen-only than on lactose-only.

The criteria C_2, and C_5 – C_8, are statistical. The theoretical values of C_2 and C_5 – C_8 are given in statistical tables [17]. Fisher coefficient for C_2 (Statistic λ) is $F_T^{\lambda}(2,11) = 4.04$. Fisher coefficients ($C_5$ and C_6) are $F_T(11,2) = 19.40$, and for correlation coefficients (C_7 and C_8) the tabular value is $R_T^2(11) = 0.684$ for level of significance $\alpha = 0.01$.

Table 2. Criteria for models validation for lactose-only

Models	$C_1(S)$	$C_2(S)$	$C_3'(X)$	$C_4(S)$	$C_5'(X)$	$C_6(S)$	$C_7'(X)$	$C_8(S)$
$M_1(S)$	13.8827	151.8377	0.2262	0.5030	1.0125	1.1676	0.9864	0.9754
$M_2(S)$	9.6736	**204.1643**	0.2058	**0.6288**	1.0598	**1.0253**	**0.9744**	0.9920
$M_3(S)$	10.6736	**151.3657**	0.1982	0.3879	1.0220	1.1364	**0.9869**	0.9819
$M_4(S)$	9.2521	175.7246	0.1960	0.3883	1.0471	1.0591	0.9790	0.9894
$M_5(S)$	14.0315	151.7896	0.2274	0.5067	1.0124	1.1690	0.9863	0.9752
$M_6(S)$	13.9697	151.9010	0.2270	0.5051	1.0129	1.1684	0.9863	0.9753
$M_7(S)$	13.9765	152.1378	0.2272	0.5045	1.0130	1.1681	0.9864	0.9752
$M_8(S)$	13.8925	151.7663	0.2262	0.5036	1.0128	1.1679	0.9864	0.9754
$M_9(S)$	**14.1648**	151.9544	**0.2289**	0.5087	**1.0118**	**1.1698**	0.9863	**0.9749**
M*$_{10}$*(S)	**6.0574**	184.7091	**0.1641**	**0.1845**	**1.0694**	1.0313	0.9853	**0.9944**

Table 3. Criteria for model validation for oxygen-only

Models	$C_1(C)$	$C_2(C)$	$C_3''(X)$	$C_4(C)$	$C_5''(X)$	$C_6(C)$	$C_7''(X)$	$C_8(C)$
$M_1(C)$	0.6734	**154.5335**	0.1612	0.5992	1.0127	1.0174	**0.9992**	0.9986
$M_2(C)$	1.5593	163.0421	0.1864	**0.9618**	1.0187	1.0155	**0.9988**	**0.9963**
$M_3(C)$	1.0059	161.9099	0.1748	0.8047	1.0141	1.0203	0.9991	0.9978
M*$_4$*(C)	**0.6727**	156.9493	**0.1596**	0.6473	**1.0115**	1.0223	**0.9992**	**0.9988**
$M_5(C)$	1.1461	160.2555	0.1818	0.2635	1.0174	1.0178	0.9989	0.9975
$M_6(C)$	1.4423	**172.3611**	**0.2021**	0.2539	1.0223	1.0235	0.9989	0.9969
$M_7(C)$	**1.6108**	169.1571	0.1941	0.2713	1.0195	**1.0286**	0.9989	0.9965
$M_8(C)$	1.1806	165.3167	0.1869	**0.2492**	1.0218	1.0164	0.9989	0.9975
$M_9(C)$	1.1256	159.8748	0.1812	0.2646	1.0176	1.0169	0.9989	0.9975
$M_{10}(C)$	1.0604	163.3446	0.1855	0.2611	**1.0226**	**1.0111**	0.9990	0.9978

Table 4. Intervals of variation of lactose-only and oxygen-only criteria

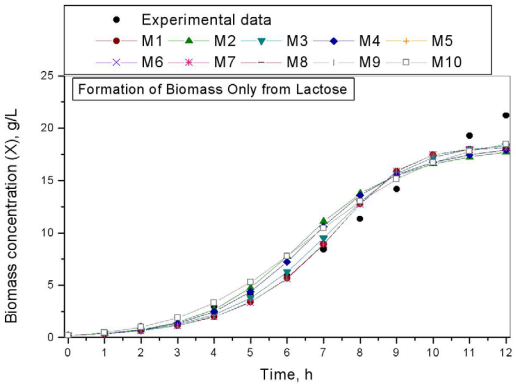
Lactose-only			Oxygen-only		
Criteria	$C_{i\min}$	$C_{i\max}$	Criteria	$C_{i\min}$	$C_{i\max}$
$C_1(S)$	6.0574	14.1648	$C_1(C)$	**0.6727**	1.6108
$C_2(S)$	**151.3657**	204.1643	$C_2(C)$	154.5335	172.3611
$C_3'(X)$	0.1641	0.2289	$C_3''(X)$	**0.1596**	0.2021
$C_4(S)$	**0.1845**	0.6288	$C_4(C)$	0.2492	0.9618
$C_5'(X)$	1.0118	1.0694	$C_5''(X)$	**1.0115**	1.0226
$C_6(S)$	1.0253	1.1698	$C_6(C)$	**1.0111**	1.0286
$C_7'(X)$	0.9744	0.9869	$C_7''(X)$	**0.9988**	0.9992
$C_8(S)$	0.9749	0.9944	$C_8(C)$	**0.9963**	0.9988

The criterion $C_2 > F_T^{\lambda}(2,11) = 4.04$, the experimental Fisher coefficients, criteria (C_5 and C_6) < $F_T(11, 2)$ = 19.40, and the experimental correlation coefficients criteria (C_7 and C_8) > $R_T^2(11) = 0.684$.

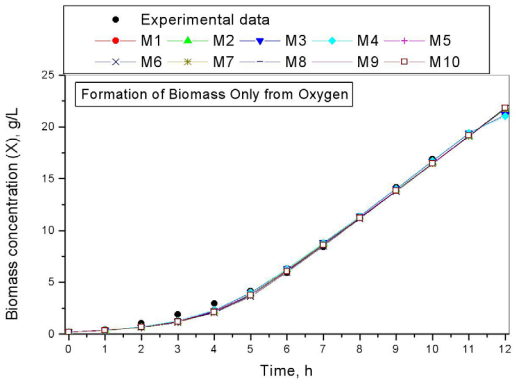
From the presented results (Tables 2, 3, and 4) it can be seen that with regard to the validation criteria (C_2, C_5 – C_8) all growth rate models, depending only on lactose or oxygen, are adequate and each of them can be used for modelling.

The kinetic parameters (due to artificial separation) of the individual growth rate models are not shown in this part of the work. They differ significantly from each other. For example, in the study on lactose-only, the maximum growth rate is $\mu_m \approx 1.0$, and on oxygen only: $0.60 \leq \mu_m \leq 0.74$. For this reason, no significant conclusions can be drawn from their values.

Fig. 1 shows the results for the biomass formation depending on lactose-only or oxygen-only.



(a)

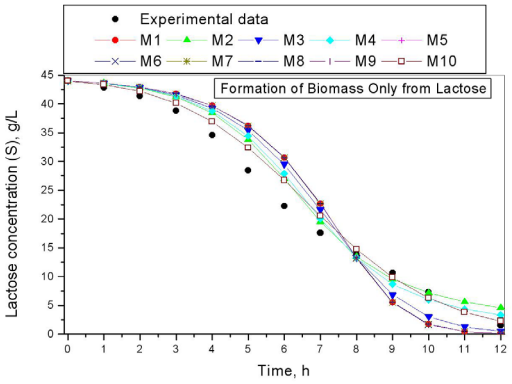


(b)

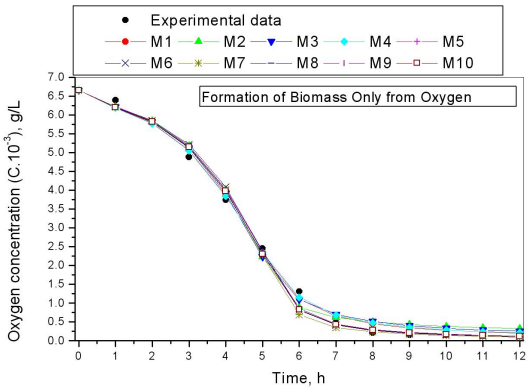
Fig. 1. Formation of biomass: a) Formation of biomass from lactose-only, b) Formation of biomass from oxygen-only.

Fig. 1a shows that the biomass concentration depends only on lactose present and there is a discrepancy between the experimental and simulated data for the kinetic variable. Fig. 1b shows that the biomass concentration depends only on oxygen present where is a different situation. All investigated growth rate models describe very well the experimental values of the kinetic variables of the process.

Fig. 2 shows the results of lactose or oxygen concentration for models (Eqns. 1) and (Eqns. 2).



(a)



(b)

Fig. 2. Lactose and oxygen concentration models: (a) Lactose concentration, (b) Oxygen concentration.

Figs. 2a and 2b show a situation similar to the reasoning made for Figs. 1a and 1b. The modelling of the lactose concentration model (Eqns. 1) shows a relatively lower correspondence between the experimental and simulated results compared to the results obtained by model (Eqns. 2), shown in Fig. 2b.

Finally, if we look formally at choosing a lactose-only growth rate model, we will notice (Table 2) that the model with the best results (criteria $C_1(S)$, $C_3'(X)$,and $C_4(S) \rightarrow$ min, and $C_8(S) \rightarrow$ max) is that of $M_{10}(S)$. Therefore, we choose the *Han-Levenspiel* model as the most suitable for modelling growth rate only from lactose.

Regarding the formal choice of growth rate model only from oxygen (Table 3), the best results by most criteria has the model $M_4(C)$. For this

model we have: $C_1(C)$, $C_3''(X)$ and $C_5''(X) \rightarrow$ min, and criteria $C_7''(X)$ and $C_8(C) \rightarrow$ max. Therefore, a logical choice for the oxygen-only growth rate is the *Moser* model.

CONCLUSIONS

In this work, ten unstructured models in batch cultivation of *Kluyveromyces marxianus var. lactis* MC 5 yeast were studied. For the first time in modelling this process two kinetic models were used, separately for lactose and oxygen. The comparison of the obtained results with the statistical criteria: statistics λ, Fisher's function and the correlation coefficient, showed that all the ten models are adequate and can be used to model the process depending on lactose-only or oxygen-only.

The formal choice of growth rate model showed that this is the *Han-Levenspiel* model for lactose-only, and the Moser model for oxygen-only.

In the next part of the work, the PROMETHEE II method will be applied to finally determine the best models for lactose and oxygen. These models will be modelled and studied.

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