Journal of International Scientific Publications: Materials, Methods and Technologies

Volume 8, ISSN 1314-7269 (Online), Published at: http://www.scientific-publications.net

MODELLING OF BATCH CULTIVATION OF *SACCHAROMYCES CEREVISIAE* USING DIFFERENT MIXING SYSTEMS

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Abstract

Mathematical models in different mixing conditions (impulse and vibromixing) in a Saccharomyces cerevisiae batch cultivation are presented in this work. Six models were investigated for the following specific grown rate: Monod, Aiba, Andrews, Haldane, Luong, and Edward. For the parameter identification, we considered the worst observed error for all experiments as an objective function. This approach is a special case of multi objective parameter estimation problems so that the parameter estimation problem becomes a min–max problem. The obtained results (correlation quotients, Fisher function, relative error and statisticsλ) show all models for the specific grown rates are adequate and they can be used for modelling of the specific grown rate for the different mixing systems. However, the best statistical indicators Luong have for the model for impulse mixing and Haldane model for the vibromixing, and they will be used in a model the process of mixing the two systems.

Key words: *specific grown rate, different mixing systems, min–max problem, modelling*

1. INTRODUCTION

Problems of mass exchange in the liquid-cell system have been investigated for decades. There is enormous literature on the optimisation of aeration and mixing conditions, the internal design of bioreactors, etc., which accounts for hundreds of references annually [7, 9-11]. The results of our studies, considered in this field, show that the intensive conditions of aeration and mixing inevitably lead to turbohypobiosis [4].

The deformation damage of cells in the intensively mixed zones proved to be much more dangerous than the insufficient mass exchange in the so-called dead zones of bioreactors. For simulation of these situations, a special bioreactor design EDF5-30 was developed to provide producers sensitive to deformation forces with even mixed cultivation conditions. For process control, specific instruments, BIO-3 and SIMD, were developed (www.bioreactors.net).

BIO-3 [8, 9, 11] allowed the control of all conventional parameters: temperature, pH, pO_2, gas flow rate, shaft rotational speed, etc. SIMD measured the kinetic energy of flow fluctuations (local stirring intensity ε, $w \cdot m^{-3}$).

It has been found that the mixing and/or aeration intensity and the limiting concentration of the substrate contribute alternatively (within reasonable ranges of variation) to the efficiency of bioreactors supplied with a gas mixture (air, nitrogen and oxygen) for pO_2 stabilisation. However, for optimal cultivation performance, each specific system (culture + bioreactor) requires individual adjustment and optimisation of the cultivation conditions.

In the [12] an alternative concept of the global models, namely *functional state modelling*, has been used. In this approach, the whole process is decomposed to *functional states*, each of which is described with a local model. The general disadvantage of this approach there are no clear criteria with that the process to be understood in which phase is, also the great number of coefficients in the model, and it is not suitable for optimization and optimal control.

In the present work a global model for modelling of a process of different mixing types of the *Saccharomyces cerevisiae* cultivation is suggested.

2. MATERIAL AND METHODS

The task of monitoring and control in fermentation processes is determined to a great extent by the potentialities of the control system. The typical potentialities of fermentation control are commonly included in the basic configuration of commercially available bioreactor controllers. Thereby, the control of the main parameters,

namely, temperature, pO_2, *pH*, foam, overpressure could be ensured, and the possibilities of the control of different parameters can be relatively wide. For the purpose of research or technological development, it is often necessary to realise the process control tasks, which are not so typical. In these cases, the controller must be flexible enough, ensuring relatively easy and quick adaptation of the program to the particular fermentation needs.

It becomes increasingly urgent nowadays to ensure the conditions of good manufacturing practice (GMP) for commercial fermentations. It means that, for fermentations also at the research stage, from the viewpoint of control registration, conditions must be ensured, which are as far as possible approximated to GMP. These conditions mainly apply to the user access control and differentiation, as well as the registration of all events, including also alarm notification and the operator's activities registration. To ensure this, the process registration and control computerised program (SCADA), devised in compliance with the corresponding requirements, should be used.

2.1. The design conception of SCADA

A computerised fermentation monitoring and control systems were developed according to the requirements of 21 CFR Part 11 (document of US Food and Drugs administration). The software is based on the ARC Informatique PC Vue industrial SCADA development package. The applications of these principles promote the performance of the conditions of GMP.

The software provides all standard SCADA functions, and ensures the audit trail of user actions, where the time of login/logout, set point changes, setting on/off of executive devices, start/stop of the process, etc. are fixed. The access to operations is allowed only for authorised users. The access level of each operator is defined through passwords.

The program drivers ensure communications between the software and many popular PLCs, for example, Siemens Simatic, Schneider Electric Quantum, General Electric Fanuc, etc. The communications can be provided also with the help of an OPC server, which gives the possibility to connect software with control units in a wide range.

2.1.1. pO_2 *control by using cascade control*

A dissolved oxygen tension, pO_2 or DOT (sometimes marked as the dissolved oxygen concentration, DOC) is a useful parameter to control aerobic processes. In BIO-3, pO_2 can be controlled in one of the following ways: stirrer rotational speed, air flow, oxygen enrichment, substrate feeding, gas mixing and pressure (overpressure). All these pO_2 control variants can be included in the cascade control. The cascade control functions according to the following rules:

1. pO_2 control is started with the first cascade. The process is controlled in the current cascade until the limits of the controlled elements are not achieved. If the limits are achieved, then the control is continued with the next or previous cascade after expiring of the "cascade delay" time. The transition direction (to the next or previous cascade) depends on the trend of the pO_2 varying dynamics and the limit achieved. The transition to the next cascade is not possible, if the current cascade is the last, and also the transition to the previous cascade is not possible if the current cascade is the first.
2. The cascade can be paused or stopped. If the cascade pauses, the current control variable is "frozen" until the process continues again. If the cascade is stopped, then the pO_2 control is also stopped. The control variables return to the starting conditions, and the process is started from the first cascade.
3. The concrete cascade process starts with the defined value of the corresponding controlled parameters. This value corresponds to one of the limits (these are defined according to Table "Cascade control conditions"). In the next cascade, the previous controlled parameter acts with the last limit value. This value of this parameter remains in all next cascades. The control parameters of all included cascades have starting values in every other cascade until the other limit of this parameter is not achieved. If the other limit of the controlled parameter is achieved, then this is the value of the controlled parameter in other cascades.

For the current fermentations, the following 3 cascades were used:

1. Stirrer rotational speed;
2. Oxygen enrichment;
3. Substrate feeding.

The using of an oxygen enrichment instead of gas mixing is reasoned, if it is necessary to add oxygen. This variant is more feasible technically and economically than gas mixing. The control in each cascade is carried out in the following way:

1. *Stirrer rotational speed:* $pO_2 = f(n) \sim n$, n – stirrer rotation speed, rpm.
   Control conditions:

$pO_2 < SP - DZ$	$SP - DZ < pO_2 < SP + DZ$	$pO_2 > SP + DZ$
$n \uparrow^{\text{PID}}$	n = const.	$n \downarrow_{\text{PID}}$

Limit conditions: [$n_{\min}$, $n_{\max}$]

2. *Oxygen enrichment:* $pO_2 = f(Ro_2) \sim Ro_2$, Ro_2 – ratio of oxygen valve Vo_2 opening time to the impulse period. Control conditions:

$pO_2 < SP - DZ$	$SP - DZ < pO_2 < SP + DZ$	$pO_2 > SP + DZ$
$Ro_2 \uparrow^{\text{PID}}$	Ro_2 = const.	$Ro_2 \downarrow_{\text{PID}}$

Limit conditions: [$Ro_{2\min}$, $Ro_{2\max}$]. Air flow Q_{air} = const.

3. *Substrate feeding:* $pO_2 = f(P_{feed})$ and $pO_2 \sim P_{feed}$ (Substrate portion increases pO_2). Control conditions:

$pO_2 < SP - DZ$	$SP - DZ < pO_2 < SP + DZ$	$pO_2 > SP + DZ$
$P_{feed} \uparrow^{\text{PID}}$	P_{feed} = const	$P_{feed} \downarrow_{\text{PID}}$

Limit conditions: [$P_{feed\ \min}$, $P_{feed\ \max}$]

2.1.2. On-line measurement of viscosity

When the process is monitored by a substrate feeding and especially by control of biomass concentration and quality, the role of viscosity values in the media increases notably. Therefore, within the set of conventionally controlled parameters, we considered also viscosity and developed its special indicating instrument. For this task, the possibility of *on-line* measurement was extremely important. So, for *on-line* measurement of the viscosity, special easily operated robust and sufficiently accurate measurement and control equipment has been devised. Thereby analysing the oscillations and applying the signal processing formulae, the current viscosity was determined using the correlation between the oscillation decrease and viscosity. The given sensor is sterilisable and applicable for continuous measurements during the fermentation process [10].

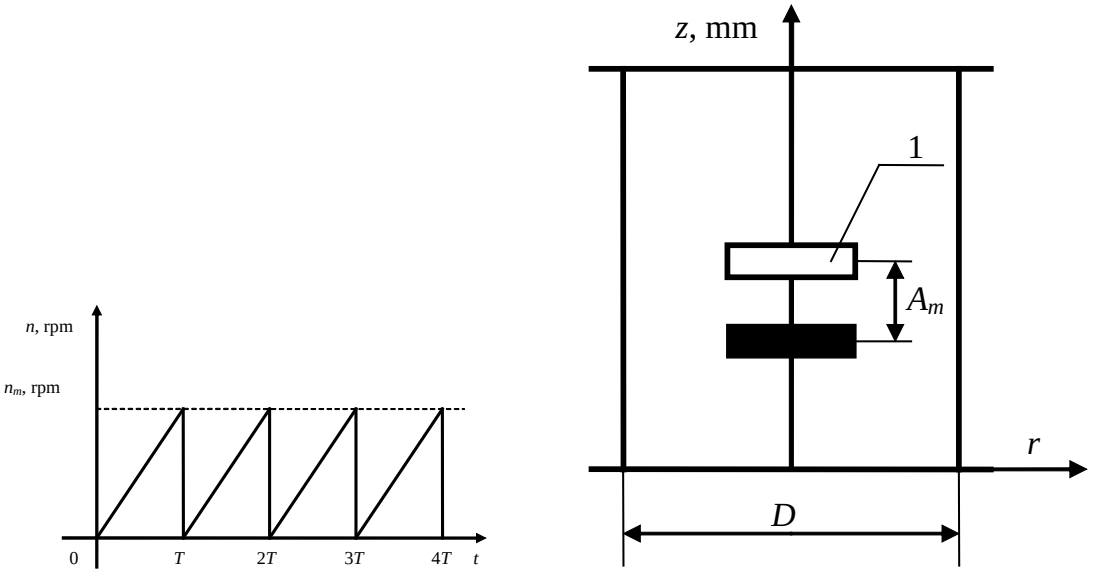
2.2. Experimental results

Two experiments were carried out in a bioreactor with the total volume 5 litres and the working volume V = 3 litres. Impulse mixing system included a double Rushton turbine with baffles. Maximum rotation speed of the stirrer is n = 260 rpm and mixing impulses with the frequency is f = 0.5 s^{-1} (Fig. 1a). Vibromixing is realised with replacing the turbine stirrer with vibrator plate – amplitude A_m= 10 mm and frequency f = 10 s^{-1} (Fig.1b).

The experiments were realised in a batch culture (2% glucose broth) of *Saccharomyces cerevisiae* in aerobic conditions (aeration – 1 L gas per 1 L broth). In the experiments of the current article, were used a laboratory bioreactor EDF-5.3, equipped with a novel upper magnetic drive, a bioprocess controller BIO-3 and a SCADA (Figure 2). Software package STATSOFT 2 (Randec Ltd.) is used for processing of experimental data.

The experimental results for the different mixing systems are shown in Table 1, where X_1, X_2, S_1, and S_2 – cell and glucose concentration for impulse and vibromixing.

In the case of impulse mixing, the classic growing curve with a plateau region is observed at the end of the process (Table 1). It can be concluded from the curve that the substrate is completely used and the cells started to die.



a) impulse mixing b) vibromixing

Figure 1. Impulse and vibromixing realised

Table 1. Experimental investigations for different mixing systems

Impulse mixing			Vibromixing	
t h	$X_1(t)$ g·l^{-1}	$S_1(t)$ g·l^{-1}	$X_2(t)$ g·l^{-1}	S_2(t) g·l^{-1}
0.0	0.89	13.80	1.20	15.75
1.0	1.17	12.30	1.43	13.95
2.0	1.59	9.24	1.79	11.97
3.0	2.43	4.70	2.31	8.72
4.0	3.19	0.60	3.02	4.77
5.0	3.20	0.10	3.99	0.63

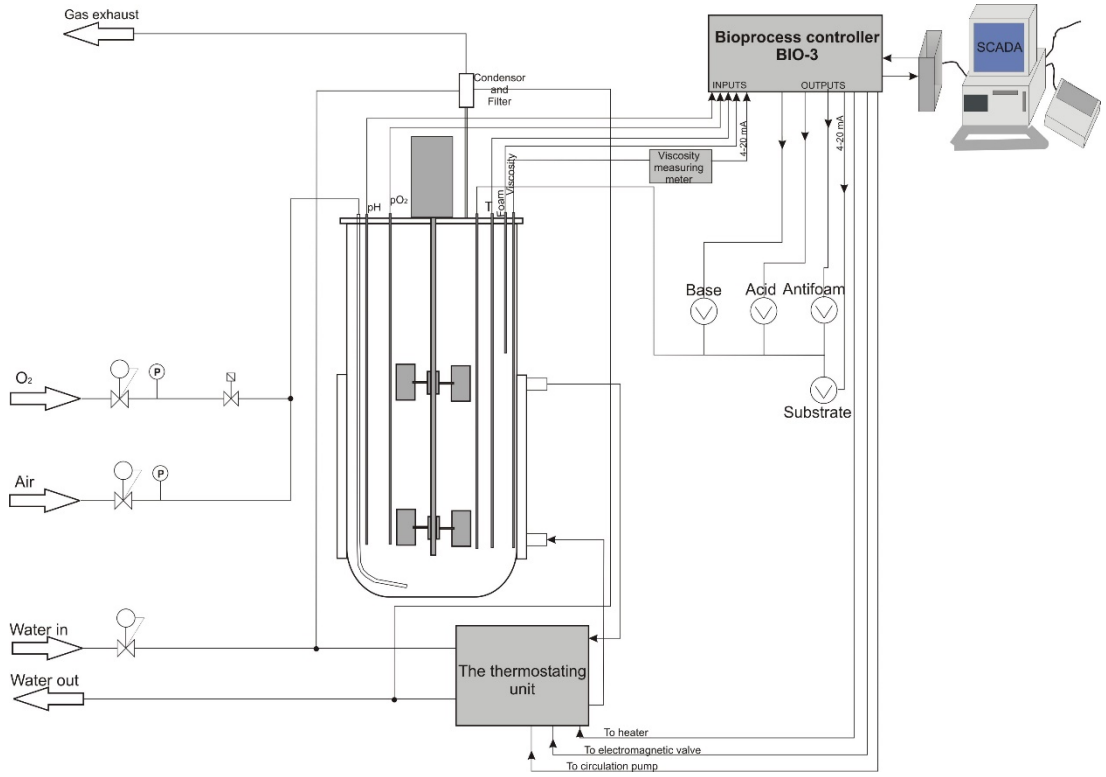


Figure 2. Schematic diagram of the fermentation process

3. KINETIC MODELS

The mathematical model of the process for the impulse and vibromixing systems is based on the mass balance equations by perfect mixing in bioreactor:

$$\frac{dX_i}{dt} = \mu_i^j X_i \tag{1}$$

$$\frac{dS_i}{dt} = -\frac{1}{Y_{i\,X/S}} \mu_i^j X_i, \quad i = 1,2; \quad j = 1,...,6 \tag{2}$$

The model for the specific grown rate is unknown, so the work test these models [3, 5]:

1. Monod model: $\mu_i^1 = \frac{\mu_m S}{K_S + S}$,
2. Aiba model: $\mu_i^2 = \frac{\mu_m S}{K_S + S} \exp(-S / K_I)$,
3. Andrews Model: $\mu_i^3 = \frac{\mu_m S}{(K_S + S)(1 + S / K_I)}$,
4. Haldane model: $\mu_i^4 = \frac{\mu_m S}{K_S + S + S^2 / K_I}$,
5. Luong model: $\mu_i^5 = \frac{\mu_m S}{K_S + S}\left(1 - \frac{S}{S_m}\right)^n$,

6. Edward model: $$\mu_i^6 = \frac{\mu_m S}{K_S + S + (S^2 / K_{SI})(1 + S / K)},$$

where: X – biomass concentration, g·l^{-1}; S – substrate concentration, g·l^{-1}; μ – specific growth rate, h^{-1}; μ_m – maximum specific grown rate, h^{-1}; t – time, h; K_S – saturation coefficient, g·l^{-1}; $Y_{X/S}$ – yield coefficient, g·g^{-1}; K_I, K_{SI} – substrate inhibition constants, g·l^{-1}; K – constant in *Edward* model; S_m – critical inhibitor concentration in *Edwar*d model, above which the reactions stops, g·l^{-1}; n – empirical constants.

The initial conditions for the impulse and vibromixing systems are:

$X_1(0)$=0.89 g·l^{-1}, $S_1(0)$=13.80 g·l^{-1}, $X_2(0)$=1.20 g·l^{-1}, $S_2(0)$=15.75 g·l^{-1} and $V(0)$ = 3 l.

3.1. Evaluation of the model parameters

The mathematical estimation of the model parameters is based on minimization of some quantity that can be calculated and that is a function of the parameters to be estimated. If the model under consideration is linear, the estimation is generally an easy task. There exists, however, there are no general theory for nonlinear parameter estimation. 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 consider the time weighted least-squares error as a criterion for each experiment. The criterion is expressed in the form [13]:

$$J_k = \frac{1}{N_S}\sum_{j=1}^{N_S} t_j \left(\frac{(X_e(t_j) - X_m(t_j))^2}{(X_{e\max} - X_{e\min})^2} + \frac{(S_e(t_j) - S_m(t_j))^2}{(S_{e\max} - S_{e\min})^2} \right) \quad (3)$$

where N_S is the number of the sampling data.

The least-squares regression sums up every observed error in (3) to the yield an objective function.

For parameter identification, we consider the worst observed error for all experiments as an objective function. This approach is a special case of multi objective parameter estimation problems so that the parameter estimation problem becomes a min–max problem [13]:

$$\min_{\mathbf{u}} J = \min_{\mathbf{u}} \max_{k} \{ J_k, k = 1, ..., N_E \} \quad (4)$$

where N_E is the number of experiments and **u** is a vector of the estimated parameters.

Now, the min–max problem can be solved by the subroutine with double precision DBCPOL from IMSL library of COMPAQ Visual FORTRAN 90 [1]. All computations have been performed on DualCore AMD Athlon II 2900 MHz computer using Microsoft Windows XP Pro Edition operating system.

3.3. Models validation

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

It is that statistic λ has $F(m, N_E - m)$ distribution [2]. Statistic λ is defined with:

$$\lambda = \frac{(N_E - m)}{(N_E - 1)} \frac{N_E}{m} \sum_{j=1}^{m} \frac{\Delta_j^2}{S_j} \quad (5)$$

where: $$\Delta_j^2 = \frac{1}{N_E}\sum_{i=1}^{N_E} \left(X_e(t_j) - X_m(t_j)\right)^2 + \left(S_e(t_j) - S_m(t_j)\right)^2,$$

$$S_j = \frac{1}{N_E - 1}\sum_{i=1}^{N_E} \left(-\Delta_{i,j}\right)^2 \Delta_j \text{ for } m = 2.$$

The relative error S_L is determined with the help of the following equation [2]:

$$S_L = \sqrt{\frac{1}{(N_E - \nu)} \sum_{j=1}^{N_E} \frac{\left(X_e(t_j) - X_m(t_j)\right)^2}{X_e^2(t_j)} + \frac{\left(S_e(t_j) - S_m(t_j)\right)^2}{S_e^2(t_j)}} \quad (6)$$

where ν is the degree of freedom.

4. RESULTS AND DISCUSSION

The developed of the abovementioned reasoning for the two mixing systems suggests us that instead a mathematical description of the specific rates of the process by global models (models of the specific rates for the all time of the cultivation) to be searched is more appropriate different relationships depending on the dependences of glucose over time to be to sought. This is confirmed also by the initial structural identification.

The structural identification of the specific rates is not made separate from the decision of the models (1)-(2). It is done simultaneously by testing of different dependencies. The results are shown in Table 2.

Table 2. Statistical results of different mixing systems and models of specific grown rate, where: 1 – impulse mixing; 2 – vibromixing. Theoretical function of Fisher $F_T(2, 4)=6.256$, theoretical function of Fisher for Statistic λ, $F'_T(4, 2)=6.944$, and theoretical correlation coefficient $R^2_T(4)=0.811$ [6]

Stat. index		R^2				F_E			
Systems		1		2		1		2	
№	Models	X	S	X	S	X	S	X	S
1	Monod	0.9982	0.9983	0.9975	0.9993	1.0284	1.0019	1.0459	1.0115
2	Aiba	0.9989	0.9991	0.9987	0.9992	1.0223	1.0009	1.0272	0.9917
3	Andrews	0.9989	0.9990	0.9988	0.9992	1.0174	1.0001	1.0268	0.9913
4	**Haldane**	0.9992	0.9993	**0.9990**	**0.9991**	1.0126	0.9990	**1.0240**	**0.9886**
5	**Luong**	**0.9995**	**0.9995**	0.9975	0.9993	**1.0065**	**0.9965**	1.0458	1.0116
6	Edward	0.9989	0.9991	0.9989	0.9992	1.0192	1.0007	1.0256	0.9901

Stat. index		S_L				Statistic λ	
Systems		1		2		1	2
№	Models	X	S	X	S		
1	Monod	0.0470	0.6611	0.0574	0.0670	115201.1	22036.0
2	Aiba	0.0353	0.5833	0.0420	0.2429	330475.9	74950.1
3	Andrews	0.0364	0.5983	0.0410	0.2435	311402.0	82004.2
4	**Haldane**	0.0297	0.5617	**0.0341**	**0.2429**	638318.4	**149171.1**
5	**Luong**	**0.0212**	**0.5259**	0.0221	0.1668	**1581810.7**	22206.4
6	Edward	0.0353	0.5831	0.0384	0.2435	343000.6	100679.0

The obtained results in Table 2 show the best statistical indexes *Luong* model has for the impulse mixing and for vibromixing – *Haldane* model. After the structural identification of model (1)-(2) have the following type:

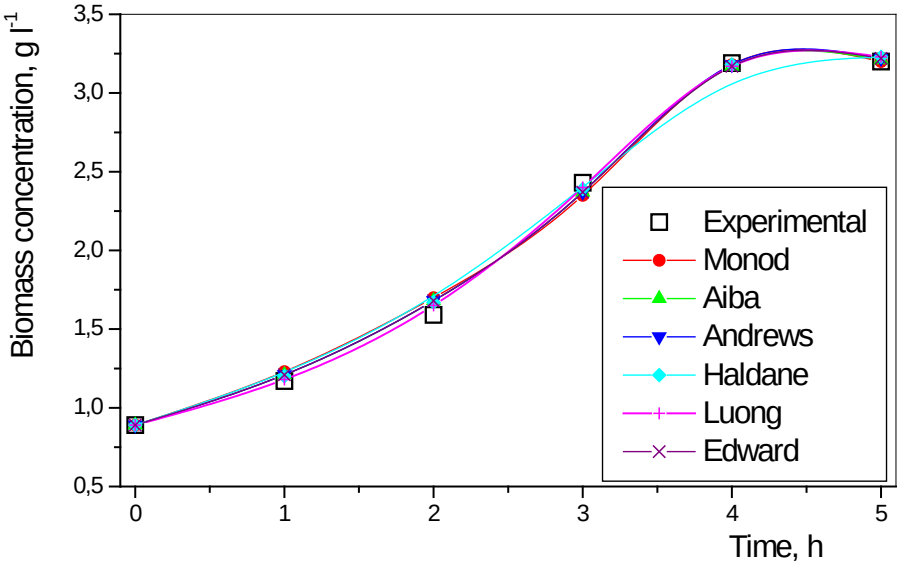
$$\frac{dX_1}{dt} = \frac{\mu_{m_1} S_1}{K_{S_1} + S_1}\left(1 - \frac{S_1}{S_m}\right)^n X_1$$
$$\frac{dS_1}{dt} = -\frac{1}{Y_1}\frac{\mu_m S_1}{K_S + S_1}\left(1 - \frac{S_1}{S_m}\right)^n X_1 \quad (7)$$

where: $\mu_{m_1} = 0.67$ h^{-1}, $K_{S_1} = 2.24$ g·l^{-1}, Y_1=0.17g·g^{-1}, n=0.66, and S_m = 20 g·l^{-1}.

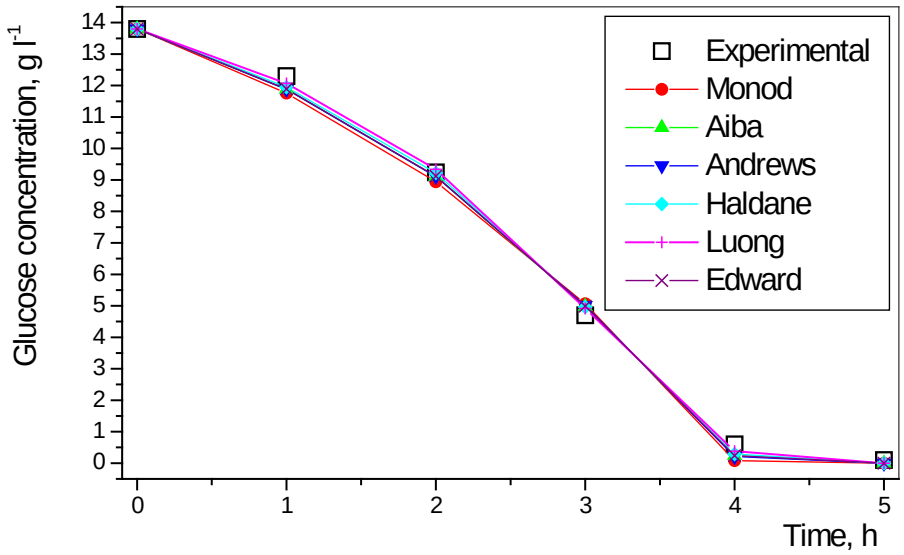
$$\frac{dX_2}{dt} = \frac{\mu_{m_2} S_2}{K_{S_2} + S_2 + S_2^2 / K_I} X_2$$
$$\frac{dS_2}{dt} = -\frac{1}{Y_2}\frac{\mu_{m_2} S_2}{K_{S_2} + S_2 + S_2^2 / K_I} X_2 \quad (8)$$

where: $\mu_{m_2} = 0.40$ h^{-1}, $K_{S_2} = 0.89$ g·l^{-1}, K_I=19.00 g·l^{-1}, and Y_2=0.18 g·g^{-1}.

The results after simulations for the biomass (X) and glucose concentrations (S) curves for the *Saccharomyces cerevisiae* cultivation using impulse and vibromixing conditions are shown in Figure 4 and Figure 5.

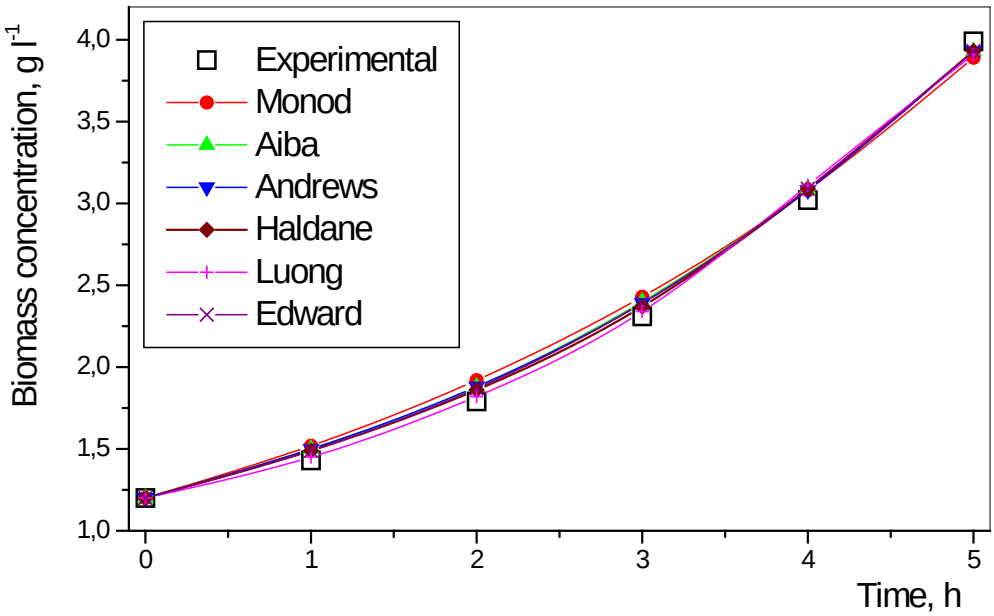


4a) Biomass concentration

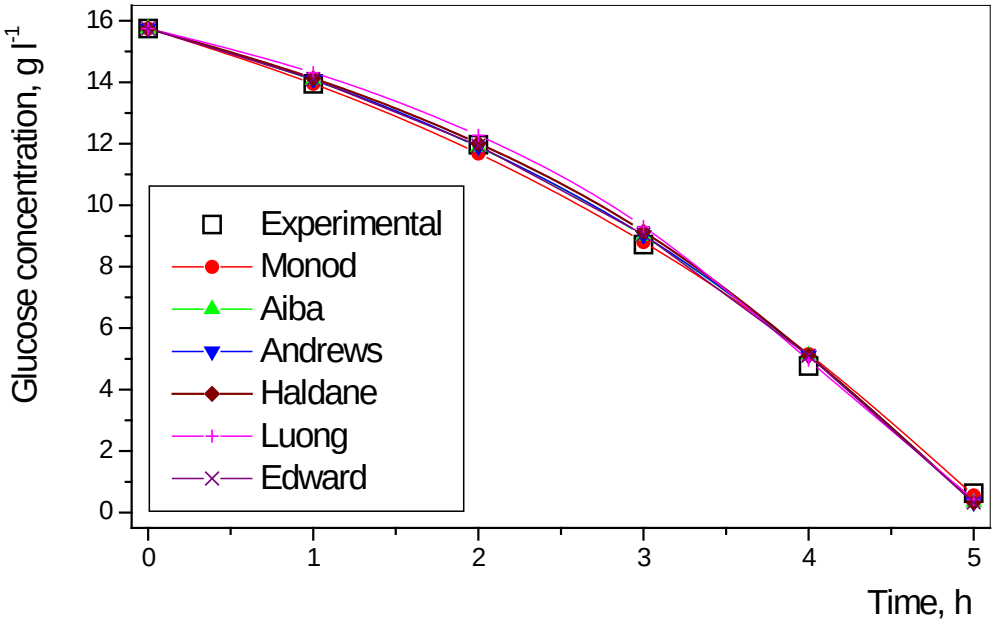


4b) Glucose concentration

Figure 4. Experimental and simulation results using impulse mixing system



5a) Biomass concentration



5b) Glucose concentration

Figure 5. Experimental and simulation results using vibromixing system

The obtained results for correlation quotients, Fisher function, relative error and statistics λ (Table 2, Figure 4 and Figure 5) show that all models for the specific grown rates $\mu(S)$ are adequate ($R^2_E > R^2_T$, $F_E < F_T$, and Statistics $\lambda > F'_T$) and they can be used for modelling of the specific grown rates for different mixing systems. However, the best statistical indicators have *Luong* model for impulse mixing and *Haldane* model for vibromixing, and they will be used in a model the process of mixing the two systems.

5. CONCLUSIONS

1. The application of a pO_2 cascade control in fermentations makes it possible to optimise the oxygen consumption at different process stages. The application of the oxygen enrichment by the pO_2 control is an economical alternative comparing with the use of a gas mixing unit. Oxygen enrichment can be used successfully enough if the oxygen impulses do not disturb the performance of the process.
2. The results of this experiments show that, for real fermentation processes, the optimisation of process control is even more important than the design of the mixing system. In comparison with the traditional continuous mixing, impulse and vibromixing decrease the ability of cells to be present in the local intensive zone in similar mixing conditions.
3. In the *Saccharomyces cerevisiae* fermentation with impulse mixing, a higher maximum growth rate is achieved than in the case of vibromixing, while a similar process yield is reached in the case of vibromixing, because, with reaching a certain density of biomass, the impulse mixing starts to affect adversely the cell growth. It means that, at a greater biomass density, it was not possible to prevent sufficiently the presence of cells in locally intensive zones.
4. The obtained results show the all models for specific grown rate are adequate. However, the best statistical indicators have *Luong* model for impulse mixing and *Haldane* model for vibromixing, and it will be used in a model the process of mixing the two systems.

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