

## **Application of Different Mixing Systems for the Batch Cultivation of the *Saccharomyces cerevisiae*. Part I: Experimental Investigations and Modelling**

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**Summary:** *Experimental investigations in different mixing conditions (impulse and vibromixing) in a *Saccharomyces cerevisiae* batch cultivation are presented in this paper. The investigation is carried out in a 5 l laboratory bioreactor (working volume 3 l). Mathematical models of the process for the two mixing systems are developed. The obtained results have shown that the models are adequate and will be used for process optimisation for the two mixing systems.*

**Keywords:** *Bioreactor, Mixing, Process control, Modelling, Cascade control, Fermentations.*

### **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, including our own publications [17-21]. The results of our studies, considered in this field, show that the intensive conditions of aeration and mixing inevitably lead to turbohypobiosis [9].

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](http://www.bioreactors.net)). BIO-3 [18, 19, 21] 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  $e$ ,  $w \cdot m^{-3}$ ).

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.

The intensifying ways were conventional mixing, mechanical vibromixing and pneumovibromixing. For estimation of the efficiency of intensifying methods used and the peculiarities of mass transfer in different conditions, physiological, biochemical and technological ( $Y_{X/S}$ ) responses of the *Saccharomyces cerevisiae* culture to different mixing types (impulse and vibromixing) were analysed.

Another important step besides the reporting of the abovementioned factors is the modelling of the process of different mixing types of *Saccharomyces cerevisiae* cultivation. The developed models will be used for optimization and optimal control of the process in different mixing systems.

### **Experimental 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.

#### *The design conception of SCADA*

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.

*pO<sub>2</sub> control by using cascade control*

Dissolved oxygen tension, *pO<sub>2</sub>* or DOT (sometimes marked as the dissolved oxygen concentration, DOC) is a useful parameter to control aerobic processes. In BIO-3, *pO<sub>2</sub>* 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<sub>2</sub>* control variants can be included in the cascade control. The cascade control functions according to the following rules:

1. *pO<sub>2</sub>* 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<sub>2</sub>* 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<sub>2</sub>* 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 use of 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:

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

Limit conditions: [*n<sub>min</sub>*, *n<sub>max</sub>*]

2. *Oxygen enrichment*:  $pO_2 = f(Ro_2) \sim Ro_2$ , *Ro<sub>2</sub>* – ratio of oxygen valve *V<sub>O<sub>2</sub></sub>* opening time to the impulse period.

Control conditions:

Air flow $Q_{air} = \text{const.}$		
$pO_2 < SP - DZ$	$SP - DZ < pO_2 < SP + DZ$	$pO_2 > SP + DZ$
$Ro_2 \uparrow^{PID}$	$Ro_2 = \text{const.}$	$Ro_2 \downarrow^{PID}$

Limit conditions:  $[Ro_{2min}, Ro_{2max}]$

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

Control conditions:

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

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

### *On-line measurement of viscosity*

When the process is monitored by substrate feeding and especially by control of biomass concentration and quality, the role of viscosity values in 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 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 [20].

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  $n = 260$  rpm and mixing impulses with the frequency  $0.5 \text{ s}^{-1}$  (Fig. 1). Vibromixing is realised with replacing the turbine stirrer with vibrator plate – amplitude 10 mm and frequency  $10 \text{ s}^{-1}$ .

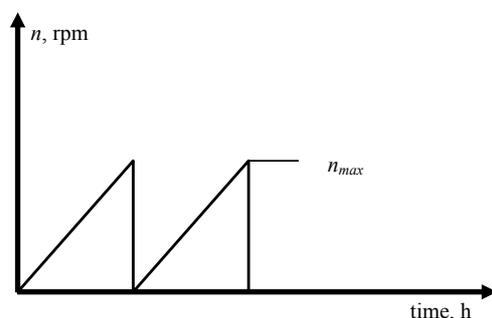


Fig. 1

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 (Fig. 2).

Software package STATSOFT 2 (Randec Ltd.) is used for processing of experimental data.

### *Experimental results*

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

vibromixing,  $g \cdot l^{-1}$ ;  $\mu_1, \mu_2, q_{S1}$ , and  $q_{S2}$  – specific growth rate and glucose consumption rate for impulse and vibromixing,  $h^{-1}$ ;  $dS_1/dt$  and  $dS_2/dt$  – glucose consumption rate for the *Saccharomyces cerevisiae* growing process,  $g \cdot l^{-1} \cdot h^{-1}$ ;  $Y_1$  and  $Y_2$  – yield of biomass from glucose growing for impulse and vibromixing.

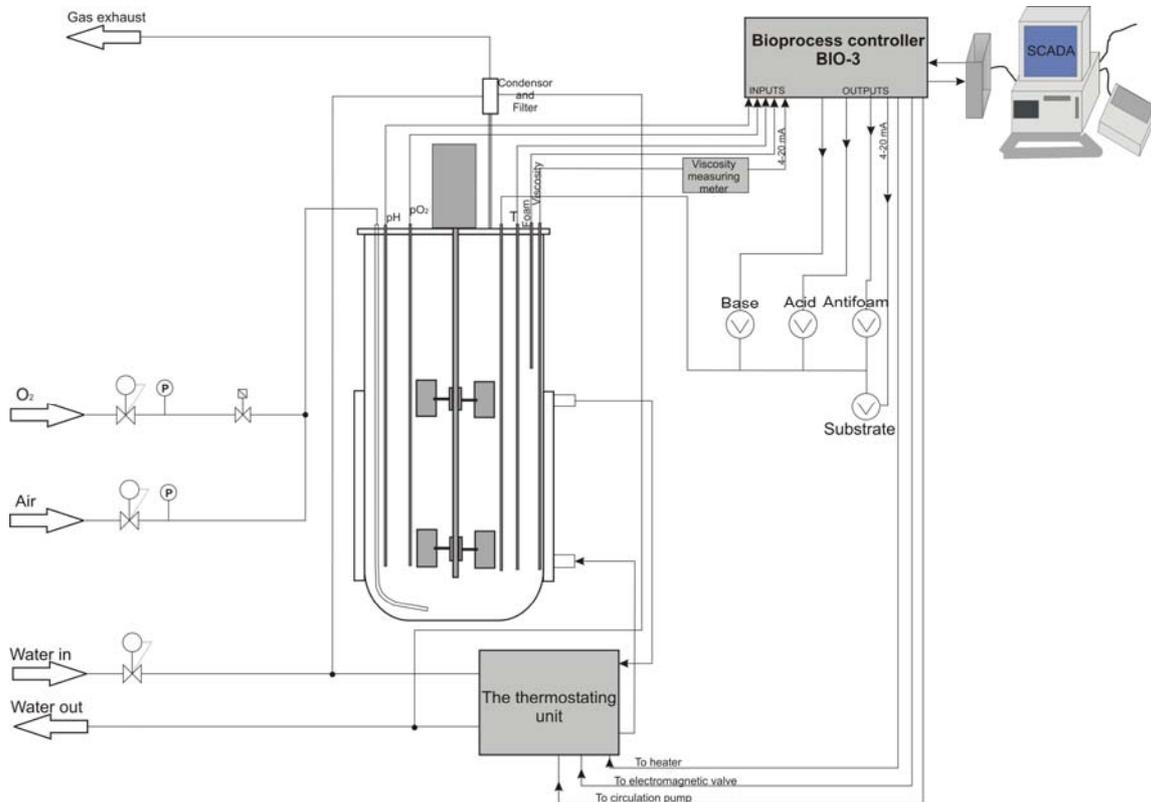


Fig. 2 Schematic diagram of the fermentation process

Table 1. Experimental investigations for different mixing systems

t h	Impulse mixing						Vibromixing					
	$X_1(t)$ $g \cdot l^{-1}$	$S_1(t)$ $g \cdot l^{-1}$	$\mu_1$ $h^{-1}$	$q_{S1}$ $h^{-1}$	$dS_1/dt$ $g \cdot l^{-1} \cdot h^{-1}$	$Y_1$ -	$X_2(t)$ $g \cdot l^{-1}$	$S_2(t)$ $g \cdot l^{-1}$	$\mu_2$ $h^{-1}$	$q_{S2}$ $h^{-1}$	$dS_2/dt$ $g \cdot l^{-1} \cdot h^{-1}$	$Y_2$ -
0	0.89	13.80	-	-	-	-	1.20	15.75	-	-	-	-
1	1.08	13.30	0.17	0.56	0.50	0.37	1.31	14.54	0.08	0.92	1.21	0.09
2	1.46	9.60	0.26	3.44	3.70	0.14	1.68	12.81	0.22	1.04	1.74	0.16
3	2.51	4.70	0.42	3.37	4.90	0.18	2.25	7.96	0.25	2.15	4.84	0.13
4	3.19	0.60	0.21	1.63	4.10	0.17	3.02	4.35	0.26	1.20	3.61	0.16
5	3.00	0.10	0.06	0.16	0.50	0.15	3.99	0.63	0.24	0.93	3.72	0.18

Fig. 3 shows the biomass and the substrate concentration curves for *Saccharomyces cerevisiae* cultivation using impulse and vibromixing systems.

If the yeast growing process is analysed using the specific growth rate of the culture, it is shown from the curves (Fig. 4a) that, using a Rushton turbine with impulse mixing, the growing of microorganisms occurs rapidly in the first three hours and then the rate of the process decreases. It may be due to decreasing of the substrate concentration and therefore a slower mass exchange. For vibromixing conditions, at the beginning of the process, the value of the specific growth rate increases in the first two hours and then the changes are minor. It is

shown better on the specific glucose consumption rate curve (Fig. 4b), where, at the end of the process, this indicator for impulse mixing processes practically reaches zero, while, for vibromixing conditions, the curve of the glucose consumption rate has increased only in the third hour of cultivation.

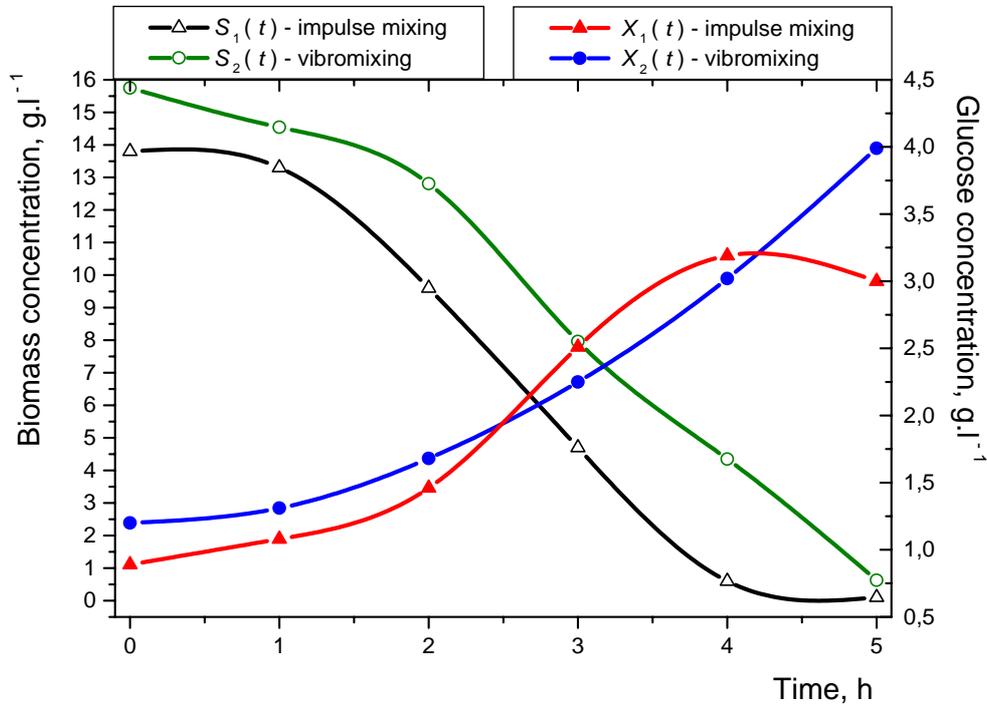


Fig. 3 Biomass and substrate concentration curves for the *Saccharomyces cerevisiae*

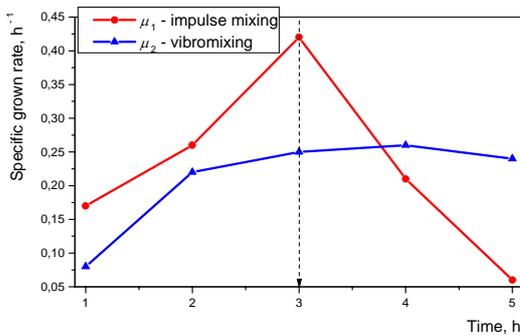


Fig. 4a Specific growth rate ( $\mu$ )

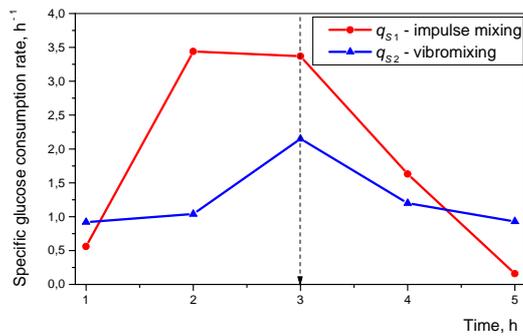


Fig. 4b Specific glucose consumption rate ( $q_s$ )

Fig. 4 Specific growth rate and specific consumption rate for the *Saccharomyces cerevisiae* growing process

In the case of impulse mixing, the classic growing curve with a plateau region is observed at the end of the process (Fig. 3). It can be concluded from the curve that the substrate is completely used and the cells started to die. This is confirmed also by the glucose consumption rate (Fig. 5a). One of the main process parameters is the product yield; in this case, it is yeast biomass. Fig. 5b shows that, for a rotary stirrer, the yield of biomass decreases twice and then remains approximately at the same level (slightly increases only in the third

hour). In the case of vibromixing, the yield of biomass increases practically slowly, in the second hour, reaches the turbine process level, and then the curves of both the processes have an approximately common tendency.

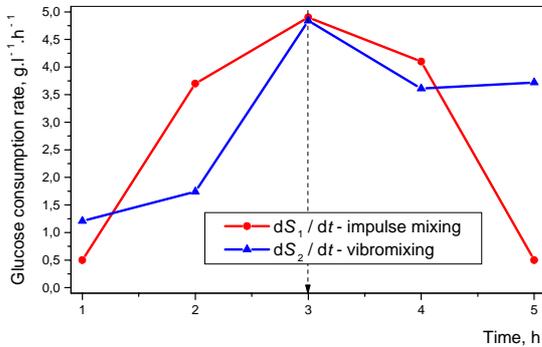


Fig. 5a Glucose consumption rate ( $dS_1/dt$ ) and ( $dS_2/dt$ )

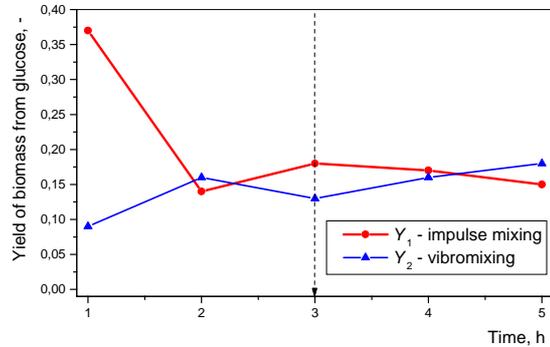


Fig. 5b Yield of biomass from glucose ( $Y_i$ ) growing

Fig. 5 Glucose consumption rate and yield of biomass from glucose growing of the *Saccharomyces cerevisiae* growing process for impulse and vibromixing

### Kinetic models

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

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

$$\frac{dS_i}{dt} = -q_{S_i} X_i, i = 1, 2 \tag{2}$$

where  $q_{S_i} = \eta_i(X_i, S_i)/Y_i$  or  $q_{S_i} = \mu_i(S_i)/Y_i$ .

The initial conditions for impulse and vibromixing is (Table 1):

$$X_1(0) = 0.89 \text{ g}\cdot\text{l}^{-1}, S_1(0) = 13.80 \text{ g}\cdot\text{l}^{-1};$$

$$X_2(0) = 1.20 \text{ g}\cdot\text{l}^{-1}, S_2(0) = 15.75 \text{ g}\cdot\text{l}^{-1}; V_1(0) = V_2(0) = 3 \text{ litres.}$$

### 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, 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 [22]:

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

where  $X_e(t_j)$  and  $S_e(t_j)$  are the measured data at  $t = t_j$ ;  $X_m(t_j)$  and  $S_m(t_j)$  are the concentrations calculated using the model;  $X_{e_{\max}}$ ,  $S_{e_{\max}}$ ,  $X_{e_{\min}}$ , and  $S_{e_{\min}}$  are the maximal and minimal measured concentrations. Here  $N_S$  is the number of the sampling data. The least-squares regression sums up every observed error in (3) to 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 multiobjective parameter estimation problems so that the parameter estimation problem becomes a min–max problem [22]:

$$\min_{\mathbf{u}} J = \min_{\mathbf{u}} \max_k \{J_k, k = 1, \dots, N_{\text{exp}}\} \quad (4)$$

where  $N_{\text{exp}}$  is the number of experiments and  $\mathbf{u}$  is a vector of the estimated parameters.

Now, the min–max problem can be solved by the random search with back step (RSBS) algorithm [16]. The algorithm was written by a FORTRAN 77. All computations have been performed on an Intel 1.8 GHz computer using Microsoft Windows XP Pro Edition operating system.

### Models validation

The model (1) and (2) validations is made by statistic  $\lambda$ . It is that statistic  $\lambda$  has  $F(m, N_{\text{exp}}-m)$  distribution [1]. Statistic  $\lambda$  is defined with:

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

where:

$$\Delta_j^2 = \frac{1}{N_{\text{exp}}} \sum_{i=1}^{N_S} \{ [X_e(t_j) - X_m(t_j)]^2 + [S_e(t_j) - S_m(t_j)]^2 \}, S_j = \frac{1}{N_{\text{exp}} - 1} \sum_{i=1}^{N_S} (-\Delta_{i,j})^2 \Delta_j, \text{ for } m = 2.$$

Specific growth rate ( $\mu$ ) and specific glucose consumption rate ( $q_S$ ) for different mixing conditions are liable to structural and parametric identification.

The structural identification of  $\mu$  and  $q_S$  is based on the statistical criteria: the Fisher quotient ( $F$ ), the correlation quotient ( $R^2$ ), and relative error ( $S_L$ ). The relative error  $S_L$  is determined with the help of the following equation [1]:

$$S_L = \sqrt{\frac{1}{(N_{\text{exp}} - \nu) \sum_{j=1}^{N_{\text{exp}}} \frac{[X_e(t_j) - X_m(t_j)]^2}{X_e^2(t_j)} + \frac{[S_e(t_j) - S_m(t_j)]^2}{S_e^2(t_j)}}} \quad (6)$$

where  $\nu$  is the degree of freedom.

## Results and discussion

### Structural identification of the specific rates

The specific rates for the different mixing systems were investigated in dependence on glucose concentration ( $S$ ) for the structure identification. The results are shown in Fig. 6.

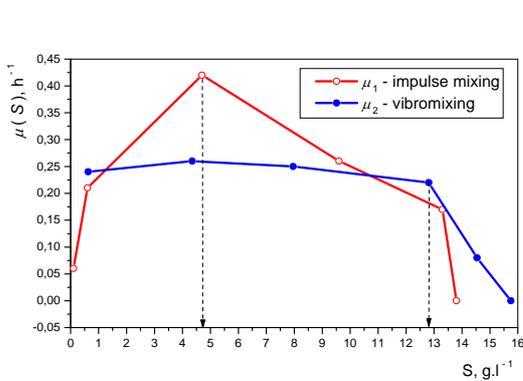


Fig. 6a  $\mu = \mu(S)$

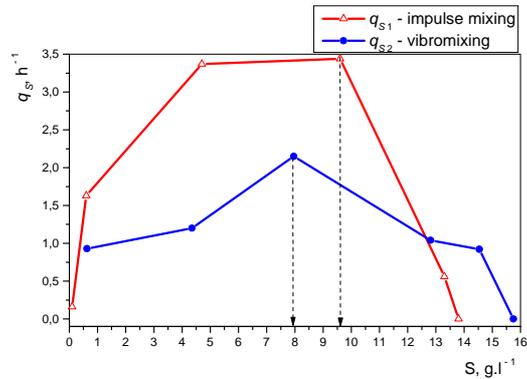


Fig. 6b  $q_S = q_S(S)$

Fig. 6 Dependence of the specific rates on the glucose concentration

The obtained experimental results (Fig. 3, Fig. 4) and trajectories of  $\mu = \mu(S)$  and  $q_S = q_S(S)$  (Fig. 6a, Fig. 6b) show in the impulse mixing from 0 h to 3<sup>rd</sup> hour and the glucose concentration change from  $S_1 = 13.80 \text{ g}\cdot\text{l}^{-1}$  to  $S_1 = 4.70 \text{ g}\cdot\text{l}^{-1}$  there is an increment of  $\mu(S)$  and  $q_S(S)$ . After that, both rates decrease. Furthermore, experimental data show (Fig. 3) that together with the synthesis of biomass and glucose consumption for impulse mixing we have and autolysis of the biomass. This fact must be taken into account in the structural identification of  $\mu_1$ .

For vibromixing, situation is similar: to 3<sup>rd</sup> hour (Fig. 4a) we have rapid growth of  $\mu_2(S)$  (Fig. 6a) almost linear reduction of  $q_S(S)$  – from  $S_2 = 15.75 \text{ g}\cdot\text{l}^{-1}$  to  $S = 12.81 \text{ g}\cdot\text{l}^{-1}$  (Fig. 6b). For  $S < 12.81 \text{ g}\cdot\text{l}^{-1}$ , we have low (almost linear) variation of  $\mu_2(S)$ . The change of  $q_S(S)$  is similar with the change of  $\mu_1(S_1)$  in impulse mixing (Fig. 6a), as we have initially increase to 3<sup>rd</sup> hour with a further decrease, as the two parts of the curve are almost symmetrical.

The developed abovementioned reasoning for the two mixing systems suggest 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 sought. This is confirmed also by the initial structural identification. The most used dependences for  $\mu$  and  $\eta$  were investigated. Only one of them adequately described the specific glucose consumption rate change for the whole cultivation period, and that was the Contois model. It will be used for description of  $\eta$  for the different mixing systems.

In this paper we will use an alternative concept of the global models, namely *functional state modelling* [5-8, 12, 14]. In this approach the whole process is decomposed to *functional states*, each of which is described with a local model. This approach was successfully applied in modelling of bacteria and yeasts [2, 4, 10, 11, 13, 15].

Experimental results show and it easy can be seen the two mixing systems have two phases. This division is conditional and not in some preliminarily criteria.

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 best dependences are defined by the statistical criteria: experimental Fisher quotient ( $F_E$ ), experimental correlation quotient ( $R^2_E$ ), and relative error ( $S_L$ ).

The dependences for the specific rates with the best statistical indexes of (1) and (2) for the two mixing systems are the Moser, Monod, and Contois models (Table 2).

Table 2. Kinetics models of the specific rates for different mixing systems

<i>Phase 1: <math>0 \leq t \leq 3</math> h and <math>S_1 \geq 4.70</math> g.l<sup>-1</sup></i>	Coefficients	<i>Phase 2: <math>t &gt; 3</math> h and <math>S_1 &lt; 4.70</math> g.l<sup>-1</sup></i>	Coefficients
$\mu(S_1) = \frac{K_1 S_1^2}{K_2 + S_1^2}$	$K_1 = 0.254$ $K_2 = 4.465$	$\mu(S_1) = \frac{K_3 S_1}{K_4 + S_1}$	$K_3 = 0.714$ $K_4 = 2.100$
$\eta(S_1) = \frac{1}{Y_{1,1}} \mu(S_1)$	$Y_{1,1} = 0.161$	$\eta(X_1, S_1) = \frac{K_5 S_1}{K_6 X_1 + S_1}$	$K_5 = 0.907$ $K_6 = 5.200$
<i>Phase 1: <math>0 \leq t \leq 3</math> h and <math>S_2 \geq 12.81</math> g.l<sup>-1</sup></i>	Coefficients	<i>Phase 2: <math>t &gt; 3</math> h and <math>S_2 &lt; 12.81</math> g.l<sup>-1</sup></i>	Coefficients
$\mu(S_2) = \frac{k_1 S_2^2}{k_2 + S_2^2}$	$k_1 = 0.161$ $k_2 = 13.276$	$\mu(S_2) = \frac{k_5 S_2}{k_6 + S_2}$	$k_5 = 0.367$ $k_6 = 0.918$
$\eta(X_2, S_2) = \frac{k_3 S_2}{k_4 X_2 + S_2}$	$k_3 = 0.312$ $k_4 = 9.280$	$\eta(X_2, S_2) = \frac{k_7 S_2}{k_8 X_2 + S_2}$	$k_7 = 0.339$ $k_8 = 1.521$

After structural identification, models (1) and (2) have the following type:

**– impulse mixing**

$$\text{Phase 1: } \begin{cases} \frac{dX_1}{dt} = \frac{K_1 S_1^2}{K_2 + S_1^2} X_1 \\ \frac{dS_1}{dt} = -\frac{1}{Y_{1,1}} \frac{K_1 S_1^2}{K_2 + S_1^2} X_1 \end{cases} \quad (7) \quad \text{Phase 2: } \begin{cases} \frac{dX_1}{dt} = \left( \frac{K_3 S_1}{K_4 + S_1} - K_d X_1 \right) X_1 \\ \frac{dS_1}{dt} = -\frac{1}{Y_{1,2}} \frac{K_5 S_1}{K_6 X_1 + S_1} X_1 \end{cases} \quad (8)$$

where  $K_d = 0.035$  and  $Y_{1,2} = 0.113$ .

A linear dependence that reflects autolysis for the biomass is included in the equation for the biomass change for *Phase 2* (8). It change is related to the biomass concentration and depends on the coefficient  $K_d$ .

**–vibromixing**

$$\text{Phase 1: } \begin{cases} \frac{dX_2}{dt} = \frac{k_1 S_2^2}{k_2 + S_2^2} X_2 \\ \frac{dS_2}{dt} = -\frac{1}{Y_{2,1}} \frac{k_3 S_2}{k_4 X_2 + S_2} X_2 \end{cases} \quad (9) \quad \text{Phase 2: } \begin{cases} \frac{dX_2}{dt} = \frac{k_5 S_2}{k_6 + S_2} X_2 \\ \frac{dS_2}{dt} = -\frac{1}{Y_{2,2}} \frac{k_7 S_2}{K_8 X_2 + S_2} X_2 \end{cases} \quad (10)$$

where  $Y_{2,1} = 0.161$ ,  $Y_{2,2} = 0.113$ .

The statistical indexes of the model are shown in Table 3. The theoretical Fisher quotient and correlation coefficient are determined for each phase for the two mixing systems and are shown in the table for the relevant degrees of freedom.

Theoretical Fisher quotient that is compared with statistic  $\lambda$  in the table is noted with  $F'_T$ .

Table 3. Statistics criteria of the models

Impulse	Variable	$R^2_E$	$R^2_T$	$F_E$	$F_T$	$S_L$	Statistic $\lambda$	$F'_T$
Phase 1	$X_1$	0.993	0.811	1.024	199.50	0.055	237495	199.50
	$S_1$	0.941		1.306		0.107		
Phase 2	$X_1$	0.994	0.811	1.011	6.26	0.018	1134609	6.94
	$S_1$	1.000		0.998		0.350		
Vibromixing	Variable	$R^2_E$	$R^2_T$	$F_E$	$F_T$	$S_L$	Statistic $\lambda$	$F'_T$
Phase 1	$X_2$	0.966	0.811	1.190	199.50	0.074	27084	199.50
	$S_2$	0.996		1.039		0.015		
Phase 2	$X_2$	0.999	0.811	1.004	6.26	0.039	236987	6.94
	$S_2$	0.998		1.010		0.267		

The results after simulations for the biomass ( $X$ ) and glucose concentrations ( $S$ ) curves for *Saccharomyces cerevisiae* cultivation using impulse and vibromixing conditions are shown in Fig. 7 and Fig. 8.

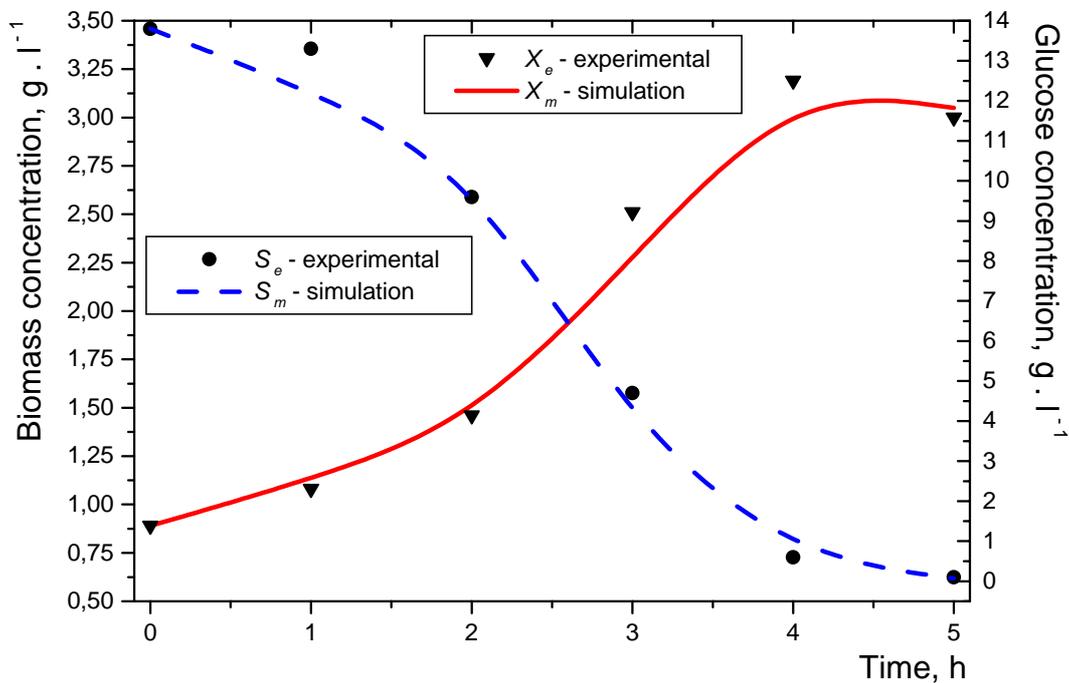


Fig. 7 Experimental and simulation results for biomass and glucose concentrations using impulse mixing system

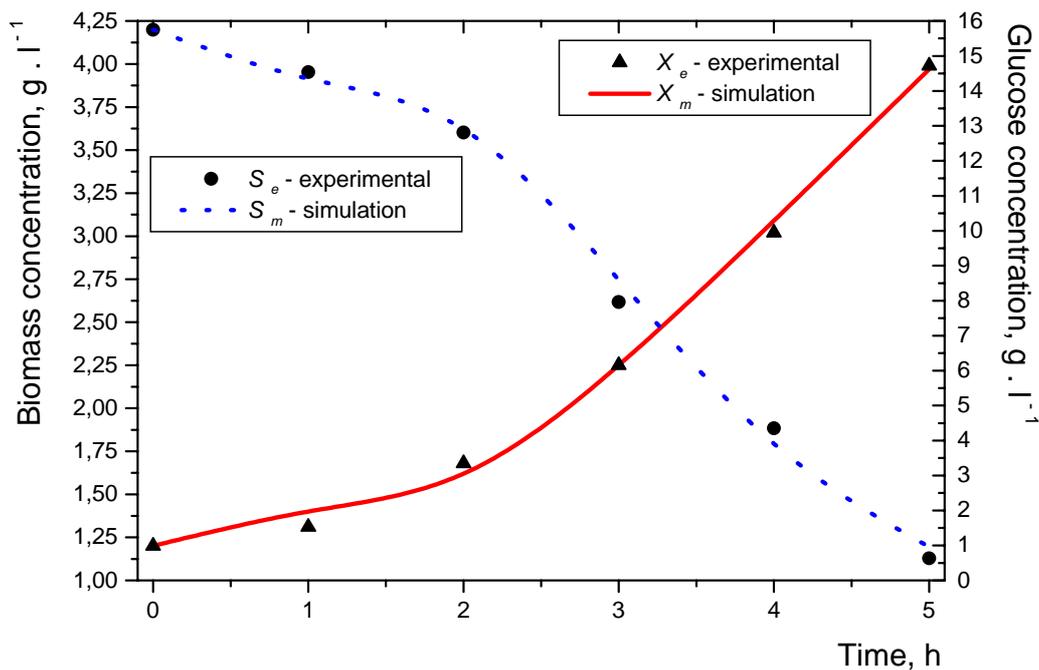


Fig. 8 Experimental and simulation results of biomass and glucose concentrations using vibromixing system

The obtained results (Fisher, correlation quotients and relative error) show that the models for the specific rates  $\mu$  and  $\eta$  are adequate ( $R^2_E > R^2_T$  and  $F_E < F_T$ ) and can be used for modelling of the specific grown rate and specific consumption of glucose.

The obtained results for models (1) and (2) (Table 3, Fig. 7, Fig. 8, and statistic  $\lambda > F'_T$ ) show that the models are adequate and can be used for optimisation and optimal control of the batch fermentation process.

## Conclusions

1. 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.
2. 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.
3. The application of  $pO_2$  cascade control in fermentation makes it possible to optimise the oxygen consumption at different process stages. The application of oxygen enrichment by  $pO_2$  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.

4. The results of this experiment show that, for real fermentation processes, the optimisation of process control is even more important than the design of the mixing system. Therefore, the second part of the study will be devoted to the optimisation and optimal process control.
5. The obtained results show the models are adequate and can be used for optimisation and optimal control of the batch fermentation process. The application of functional state modelling approach shows in this case it is better than development of a global model for the specific rates. In this work, the phase separation is conditional, not strictly. This division depends on the mixing type. In optimization and optimal control of processes and the mixing type in models will be incorporated.

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### References

1. Giridhar R., Ashok K. Srivastava (2000). Model Based Constant Feed Fed-batch *L-Sorbose* Production Process for Improvement in *L-Sorbose* Productivity, 14(4), 133-140.
2. Hristozov I., T. Pencheva, St. Tzonkov, B. Hitzmann (2005). Functional State Modelling Approach for Batch Cultivation of *Saccharomyces cerevisiae*, Chemical and Biochemical Engineering Quarterly, 19(1), 69-74.
3. Kafarov V., A. Vinarov, L. Gordeev (1985). Modelling and System Analysis Biochemical Productions, Wood Manufacture, Moscow.
4. Pencheva T., I. Hristozov, D. Huell, B. Hitzmann, St. Tzonkov (2005). Modelling of Functional States during *Saccharomyces cerevisiae* Fed-batch Cultivation. Bioautomation, 2, 8-16.
5. Pencheva T., I. Hristozov. Global Model versus Functional State Approach to Modelling of Baker Yeast Cultivations, Proceedings of Fourth National Young Scientific-Practice Session, Sofia, Bulgaria, May 19 2006, 127-132, (in Bulgarian)
6. Pencheva T., M. Petrov, T. Ilkova, O. Roeva, J. Vanags, U. Viesturs et al. (2006). Bioprocess Engineering, Chapter 6.4, U. Viesturs, S. Tzonkov (Eds.), Avangard Prima, Sofia, 204-226.
7. Pencheva T., O. Roeva, I. Hristozov, Functional State Approach to Fermentation Processes Modelling, Tzonkov St., B. Hitzmann (Eds.), Prof. Marin Drinov Academic Publishing House, Sofia, 2006.
8. Pencheva T., S. Vassileva, T. Ilkova, Y. Georgieva, B. Hitzmann, St. Tzonkov (2004). Multimodel Approach for Modelling of Biotechnological Processes, Biotechnology and Biotechnological Equipment, 18(2), 206-214.
9. Rikmanis M., A. Bērziņš, U. Viesturs (2007). Excess Turbulence as a Cause of Turbophobiosis in Cultivation of Microorganisms, CEJB, 2(4), 481-501.
10. Roeva O., K. Kosev, St. Tzonkov (2007). Mathematical Description of Functional States in *E. coli* Fed-batch Cultivation Processes, Bioautomation, 7, 34-45.
11. Roeva O., St. Tzonkov (2007). An Improvement of Functional State Local Models of *Escherichia coli* MC4110 Fed-batch Cultivation, Information Technologies & Control, V(4), 47-52.

12. Roeva O., St. Tzonkov (2008). Comparison of Global and Multiple Model Approach, *Engineering Sciences*, XLV, 3, 20-31.
13. Roeva O., T. Pencheva, St. Tzonkov, M. Arndt, B. Hitzmann, S. Kleist, G. Miksch, K. Friehs, E. Flaschel (2007). Multiple Model Approach to Modelling of *Escherichia coli* Fed-batch Cultivation Extracellular Production of Bacterial Phytase, *Electronic Journal of Biotechnology*, 10(4), 592-603.
14. Roeva O., T. Pencheva, U. Viesturs, St. Tzonkov (2006). Modelling of Fermentation Processes based on State Decomposition, *Bioautomation*, 5, 1-12.
15. Roeva O., T. Pencheva, Y. Georgieva, B. Hitzmann, S. Tzonkov (2004). Implementation of Functional State Approach for Modelling of *Escherichia coli* Fed-batch Cultivation, *Biotechnology and Biotechnological Equipment*, 18(3), 207-214.
16. Stoianov S., *Optimization of Technologies Objects, Techniques*, Sofia, 1971 (in Bulgarian).
17. Vanags J., M. Rikmanis, E. Ushkans, U. Viesturs (1990). Stirring Characteristics in Bioreactors, *AIChE J.*, 36, 1361-1369.
18. Vanags J., M. Rychtera, S. Ferzik, M. Vishkins, U. Viesturs (2007). Oxygen and Temperature Control during the Cultivation of Microorganisms using Substrate Feeding, *Engineering in Life Sciences*, 7(3), 247-252.
19. Vanags J., U. Viesturs, I. Fort (1999). Mixing Intensity Studies in a Pilot Plant Stirred Bioreactor with an Electromagnetic Drive, *Biochem. Eng. J.*, 3, 25-33.
20. Vanags J., U. Viesturs. A. Bērziņš (2008). Performance of the Cultivation of Microorganisms Using Different Mixing Systems, 18th International Congress of Chemical and Process Engineering – CHISA'08, 24-28 August, Prague, Czech Republic. Summaries 3. Systems and Technology, p. 851, CDROM full text 1-8.
21. Viesturs U. (2007). Development and Automation of Microturbulence Intensifying Systems and Biosynthesis Conditions in Bioreactors, *Bioautomation*, 7, 1-8.
22. Wang Feng-Sheng, Su Tzu-Liang, Jang 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.

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