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DIRECT SENSITIVITY ANALYSIS OF A WHITE WINE ALCOHOLIC FERMENTATION PROCESS

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ABSTRACT. Process engineering became, recently in historical terms, an extension of food engineering principles to other fields of manufacturing as the food biotechnology and beverage industry is. This article proposes a non-linear mathematical model that makes possible the simulation of the batch alcoholic fermentation of white wine. The model was developed on the basis of zone modelling principle, considering the physiological states of the yeast cells. The nonlinear mathematical model led to a good qualitative and quantitative description of the alcoholic fermentation process. The model was implemented as a Matlab S-function and the results were compared with experimental data. Further on, the mathematical model was used for the investigation of the dynamic behaviour of a batch fermentation process through direct sensitivity analysis method (DSA). The DSA of dynamic model allowed the calculus of the matrix of the sensitivity functions in order to determine the influence of the small deviations of initial state and parameters from their nominal values on the state trajectory and system output over the time.

Keywords: *alcoholic fermentation process, nonlinear mathematical model, process simulation, direct sensitivity analysis*

INTRODUCTION

Wine making is a complex ecological and biochemical process involving many interactions such as must variety, microbiota and several operations. It is often controlled empirically and traditionally. There are some factors that strongly affect the alcoholic fermentation. The most important ones are: fermentation temperature, grape juice composition, anaerobic conditions - due to CO_2

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production, low media pH, sulphur dioxide concentration level, selected yeasts inoculation and interaction with other microorganisms [1]. The models developed for these cases consequently have various domains of applications but none of them include the whole oenological aspects of the process. The majority of the models are of "knowledge-based" models type and they take into consideration a great number of phenomena that have an important effect on the kinetics of the process fermentation [2].

The review [3] presented different approaches that have been taken for "knowledge-based" models, non-physiological mathematical descriptions, behaviour prediction models and empirical models and others authors [4, 5, 6, 7] used a kinetic model that assumes a limited oxidation capacity of yeast, leading to the formation of ethanol under conditions of oxygen limitation and/or high glucose concentration. Therefore they require the estimation of many variables which are often measured with difficulty.

On the other hand, the knowledge-based models inaccurately describe the end kinetics of the fermentation process [8]. In addition, there are other models resulting from the macroscopic analysis of the process fermentation [9, 10]. These models require less estimated variables but their physiological significance is difficult to analyse. These models describe very well the evolution of the main variables specific to the alcoholic fermentation process such as the substrate and alcoholic concentrations [11].

Recently, many studies are focused on models which use artificial intelligence techniques [12, 13] thus having a high capacity of generalization and a good stability. In [14] artificial neural networks are used. In [15] a control system using two feed-forward ANN models is proposed: the first network is incorporated in the hybrid model of the process, providing values for the plant output; the second network performs the task of feedback controller in the form of a plant with inverse plant dynamics. In [16] the neural network is adapted on-line using only the dissolved oxygen measurement to record varying operating conditions while other states of the system, namely the substrate, ethanol and biomass concentrations are not measured but predicted by the adapted network.

Although real results begin to appear, there is still necessary to validate these models on a wide range of fermentation processes. In other words, a complex data-base containing information about the kinetics of the alcoholic fermentation processes has to be created and used [17] so that the approach of the modelling of these processes through artificial intelligence model-based to be consistent. For these reasons, the modelling, simulation and control of these processes is not a fully solved problem yet and still remains a challenge for the specialists in control and in wine producing technology (question: is it possible to have an overall valid model at all sort of wines?). This paper proposes a general mathematical model of the alcoholic fermentation process. A study regarding the analysis of this model on the basis of the sensitivities in relation with the initial state and the main parameters is carried out, having the goal of designing and implementing a control strategy of the alcoholic fermentation process [18].

The paper structure is as follows: after a brief introduction, in the second part the present article proposes a non-linear model for white wine alcoholic fermentation process which, besides the detailed kinetic model, involves equations corresponding to the physiological phases of yeast cells, the inhibitory effect of ethanol, heat transfer equations and the dependence of kinetic parameters on temperature. In the third part of the paper the dynamic behaviour of a batch fermentation process using the direct sensitivity analysis (DSA) is investigated. Finally, the last section is dedicated for conclusions.

RESULTS AND DISCUSSION

Result and discussion regarding the mathematical model simulation

The nonlinear mathematical model of the batch fermentation process (Table 1) used in this work contains the following equations:

- an equation for the latent phase of fermentation that describes the dependence of the phase time of the process temperature;
- the model proposed by Aiba [17] for the growing phase with the three equations of biomass, alcohol production and substrate consumption;
- the model presented by Bovée-Strehaiano [9] for the decay phase with two equations: one for the substrate consumption and the other for alcohol formation;
- an equation that describes the biomass behaviour along the phase no. 3 (the model proposed by Sipos in [19, 20]);
- an energy-balance model in which the rate of change of the medium's temperature (**dT^0/dt**) is a result of the balance between the rate of the heat generation due to fermentation and the rate of the heat transfer to the cooling medium inside the bioreactor jacket.

The model proposed by Aiba includes the inhibitory effects of the fermentation product (alcohol). In the growing phase the value of the maximum specific growth rate of the biomass corresponds to the real one. The non-physiological model proposed by Bovée and Strehaiano was chosen because it accurately describes the substrate consumption and the evolution of the alcohol concentration in the growing and decay phases. This model proposes the use of a semi empirical model in which the velocity of sugar consumption is described by a chemical law that depends on substrate and product contents. The parameters of the model are adjusted by means of non-linear programming methods, which compare model predictions with experimental data and minimise errors [19, 20]. The Bovée and Strehaiano model is capable of predicting the fermented sugar (and thus thermal planning) within an error of 3.3% [4]. Thus, the model offers a good qualitative and quantitative description of the behaviour of the alcoholic fermentation process.

Figures 1, 2 and 3 show the simulation results of the model presented in Table 1 considering the following initial values: the initial substrate concentration was 210 $g{\cdot}L^{-1}$ and the fermentation temperature was 301K.

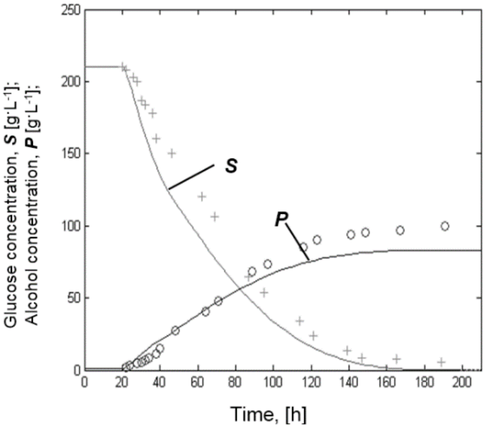

Figure 1. Evolution of glucose and alcohol concentrations; a comparison between experimental values (**o** - glucose and **+** - alcohol) and simulation results (continuous lines)

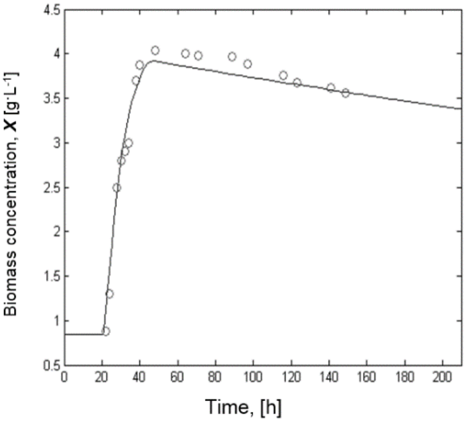

Figure 2. Comparison between the biomass simulation results (continuous line) and experimental data (o)

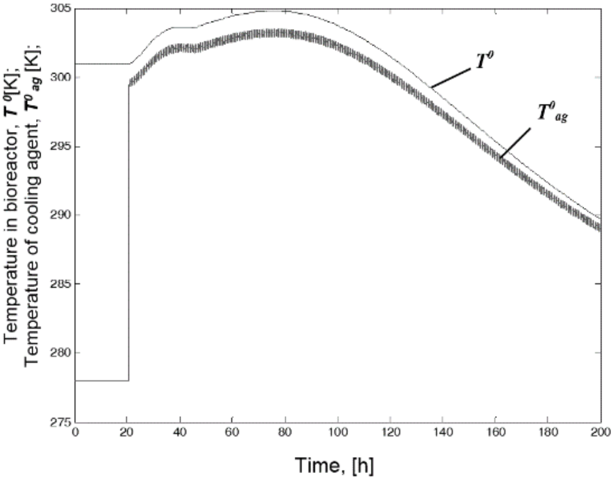

Figure 3. Temperatures of the fermentation medium and cooling agent (simulation results)

The equation of the latent phase is valid for a time interval [0, 100 h] and the model has been tested for a grape juice variety with an initial concentration of the substrate varying between 180 and 210 g·L^{-1}, a fermentation temperature between 299 and 303 K and without aeration.

DSA results and discussion

DSA with respect to the initial state

Figures 4 – 6 present the graphs of the sensitivity functions. Based on these graphs the following remarks can be drawn:

- The substrate consumption, the biomass production and the fermentation temperature present the same sensitivity with respect to the initial concentration variation of the biomass (Figure 4). The influence of this variation is significant throughout the exponential growing phase and then it decreases when the process reaches the decay phase. This influence proves that the substrate consumption depends on both viable biomass concentration and biomass growth rate.

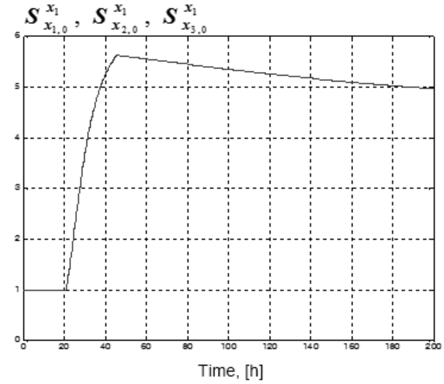

Figure 4. Graphs of the biomass sensitivity functions in relation with the variation of: biomass initial concentration, $S^{x_1}_{x_{1,0}}$ [(g·L^{-1} biomass)/(g·L^{-1} biomass)$_0$], substrate initial concentration, $S^{x_1}_{x_{2,0}}$ [(g·L^{-1} biomass)/(g·L^{-1} substrate)$_0$] and process initial temperature, $S^{x_1}_{x_{3,0}}$ [(g·L^{-1} biomass)/(K)$_0$]

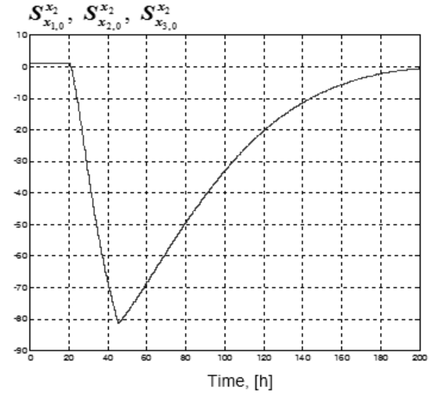

Figure 5. Graphs of the substrate sensitivity functions in relation with the variation of: biomass initial concentration, $S^{x_2}_{x_{1,0}}$ [(g·L^{-1} substrate)/(g·L^{-1} biomass)$_0$], substrate initial concentration, $S^{x_2}_{x_{2,0}}$ [(g·L^{-1} substrate)/(g·L^{-1} substrate)$_0$] and process initial temperature, $S^{x_2}_{x_{3,0}}$ [(g·L^{-1} substrate)/(K)$_0$]

- The change of the initial concentration of the substrate in relation with the three variables (substrate, biomass and fermentation temperature) reaches a maximum (negative) at the end of exponential growing phase (Figure 5). The influence exists only in this phase which is the most important one in fermentation.

The initial concentration of the substrate has a strong influence in the exponential growing phase which may lead to a modification of the fermentation duration with all the consequences that may occur. A short duration leads to a tumultuous fermentation, because the initial concentration of substrate is too lower or not all substrate is metabolized wholly through fermentation or the alcohol production rate is too high and has an inhibitory effect on the biomass development. A long duration leads to a slow fermentation, with lower alcohol concentration at the end.

- The change of the initial temperature of the fermentation over the substrate, biomass and temperature inside the bioreactor presents a positive maximum at the beginning of the exponential growing phase and after that decreases in time. The possibility that the fermentation duration may be changed is once again confirmed and so the consequences that can appear.

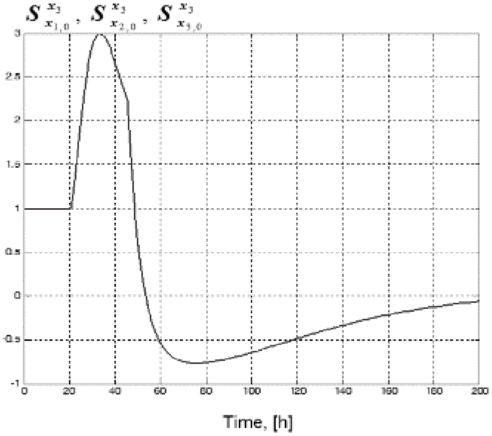

Figure 6. Graphs of the process initial temperature sensitivity functions in relation with the variation of: biomass initial concentration, $S^{x_3}_{x_{1,0}}$ [(K)/(g·L^{-1} biomass)$_0$], substrate initial concentration, $S^{x_3}_{x_{2,0}}$ [(K)/(g·L^{-1} substrate)$_0$] and process initial temperature, $S^{x_3}_{x_{3,0}}$ [(K)/(K)$_0$]

DSA with respect to the parameters of the mathematical model

The parameters' sensitivity (Figures 7–9) highlights that the process kinetics (substrate consumption, biomass production and fermentation temperature) is strongly influenced by the modification of the two kinetics parameters (***A*** and ***E$_a$***), whose values are frequently determined with uncertain precision. The alcoholic batch fermentation of white wine being a process controlled by temperature, the heat transfer area modification lead to changes in the fermentation conditions and, as a consequence, these can affect the process duration and the wine quality and characteristics.

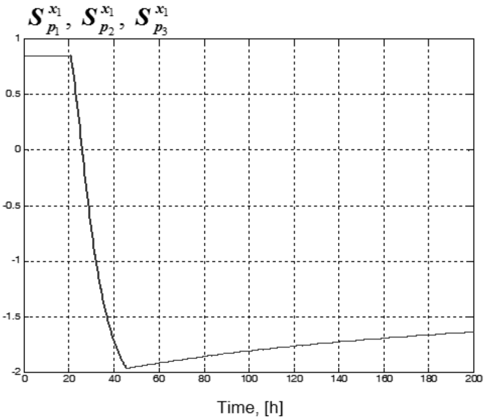

Figure 7. Graphs of the biomass sensitivity functions in relation with the variation of parameters: $S_{p_1}^{x_1}$ [(g·L^{-1} biomass)/(h^{-1})], $S_{p_2}^{x_1}$ [(g·L^{-1} biomass)/(K)] and $S_{p_3}^{x_1}$ [(g·L^{-1} biomass)/(m^2)]

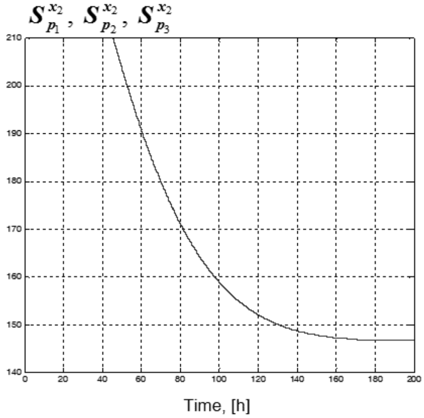

Figure 8. Graphs of the substrate sensitivity functions in relation with the *variation* of parameters: $S_{p_1}^{x_2}$ [(g·L^{-1} substrate)/(h^{-1})], $S_{p_2}^{x_2}$ [(g·L^{-1} substrate)/(K)] and $S_{p_3}^{x_2}$ [(g·L^{-1} substrate)/(m^2)]

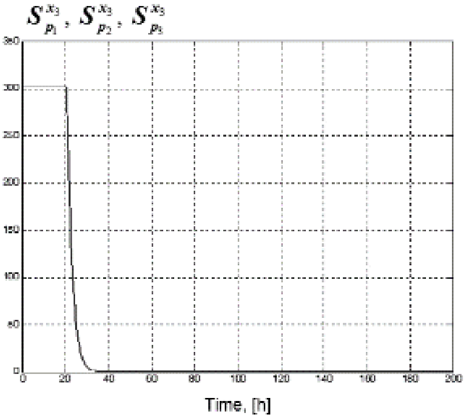

Figure 9. Graphs of the process initial temperature sensitivity functions in relation with the variation of parameters: $S_{p_1}^{x_3}$ [(K)/(h^{-1})], $S_{p_2}^{x_3}$ [(K)/(K)] and $S_{p_3}^{x_3}$ [(K)/(m^2)]

CONCLUSIONS

The dynamic model presented in this paper enables the prediction of the evolutions and final concentrations of biomass, alcohol and substrate during a batch alcoholic fermentation. The model involves equations corresponding to the physiological phases of yeast cells, the inhibitory effect of ethanol, heat transfer equations and the dependence of kinetic parameters on temperature.

The model was validated through four experiments considering two different temperatures and two different initial concentrations of substrate for the same must. So, by this general model, the possibility to simulate real, oenological aspects that can appear during the alcoholic fermentation process it was obtained. Also, based on this model, the direct sensitivity analysis was realized. The sensitivity functions matrix calculated with DSA allowed mono and multivariable estimation of the influences of input variables and parameters. Considering the DSA it were proved that the initial concentrations of the biomass, the changes of the initial concentration of the substrate and initial values of temperature have a strong influence in the exponential growing phase, which is the most important one in fermentation because the maximum growth rate of the selected strain depends on operating conditions which must be known exactly.

The parameters' sensitivity analysis pinpointed the process' kinetics aspects as are the strongly influences of the fermentation process by the modification of the two kinetics parameters (the pre-exponential factors in Arrhenius' equation and the activation energies), whose values are frequently determined with uncertain precision.

The results obtained in this study will allow the possibility to develop state observers (substrate, biomass and product concentrations) using temperature measurements and to design an appropriate control system.

EXPERIMENTAL

The article proposes a complex nonlinear wine fermentation model based on previous researches of the authors [19, 20] followed by a sensitivity analysis designed to develop state observers (substrate, biomass and product concentrations) using only temperature measurements and to configure the control scheme of the process.

Experimental conditions

To evaluate the total fermentation yield losses under different operating conditions, four experiments were carried out and based on the data obtained within these experiments, a mathematical model was proposed. The strain and the culture medium, the equipment and the experimental conditions together with the measurements of the fermentation parameters were presented by Sipos and co-workers [19, 20]. For the experiments the *Saccharomyces cerevisiae* YEPD wine yeast was used, being seeded on a culture medium with the following composition: 5 $g\cdot L^{-1}$ KH_2PO_4, 2 $g\cdot L^{-1}$ $(NH_4)_2SO_4$, 0.4 $g\cdot L^{-1}$ $(MgSO_4)\cdot 7H_2O$, 1 $g\cdot L^{-1}$ yeast extract, 50 $g\cdot L^{-1}$ glucose and Mauzac must (sterilized through flash pasteurization). The sugar content of the grape must was supplemented with sucrose up to 180 $g\cdot L^{-1}$ and 40 $mL\cdot h^{-1}\cdot L^{-1}$ tiaminol were added. The SO_2 content reached 50 mg·L^{-1} and the pH was adjusted at 3.8 mg·L^{-1} H_3PO_4. Both the fermentation medium and the bioreactor were autoclaved for 20 minutes at 393 K. A New-Brunswick continuously stirred bioreactor equipped with pH and temperature sensors was used.

The following operating conditions were:

- Working volume – 8 L;
- Temperature – 291 K and 301 K;
- Stirring speed – 150 rpm;
- pH – 3.8;
- Influent glucose concentrations – 180 g·L^{-1} and 210 g·L^{-1};
- Without aeration, the necessary oxygen was dissolved in must.

The mathematical model

The mathematical model of the alcoholic fermentation process was determined on the basis of the approach of the zone modelling principle, taking into consideration the evolution of the viable biomass ($X_v(t)$). Based on the analysis of the phenomenological aspects of the alcoholic fermentation process, the evolution of $X_v(t)$ was divided in three parts (Figure 10) as follows:

- latent phase (1);
- growing phase (2);
- decay phase (3).

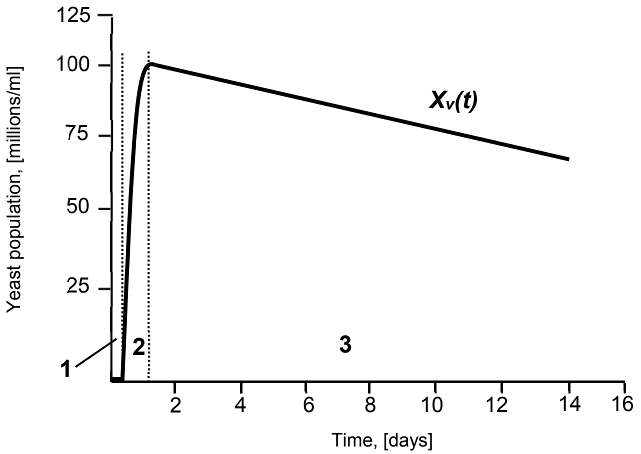

Figure 10. Evolution of the viable biomass concentration $X_v(t)$

Table 1 presents the equations of the model. The parameters are adjusted through the non-linear programming method, which compares the model predictions with experimental data and minimises the errors.

Table 1. The model of the alcoholic fermentation process

Current phase	**Model equations**
Kinetic model	
Latent phase [19, 20]	$t_{lat} = \frac{a}{T^0} + b$
Exponential growing phase [17]	- biomass: $\frac{dX}{dt} = \mu_{\max} \cdot \left(\frac{S}{K_S + S} \right) \cdot e^{-K_p \cdot P} \cdot X$; $\mu_{\max} = A_1 \cdot e^{-\frac{E_{a1}}{R \cdot T^0}} - A_2 \cdot e^{-\frac{E_{a2}}{R \cdot T^0}}$ - alcohol: $\frac{dP}{dt} = q_{p\max} \cdot \left(\frac{S}{K_{SP} + S} \right) \cdot e^{-K_{pp} \cdot P} \cdot X$ - substrate: $\frac{dS}{dt} = -\left(\frac{1}{Y_{XS}} \cdot \frac{dX}{dt} \right) - \left(\frac{1}{Y_{PS}} \cdot \frac{dP}{dt} \right)$
Decay phase [9, 19, 20]	- biomass: $\frac{dX}{dt} = f \cdot X \cdot k$; $k = A \cdot e^{-\frac{Ea}{R \cdot T^0}}$ - alcohol: $P = P_0 + \eta \cdot (S_0 - S)$ - substrate: $\frac{dS}{dt} = -k \cdot S^{\alpha} \cdot P^{\beta}$
Energetic model	
All phases [18, 19]	- for the bioreactor: $\frac{\Delta Hr \cdot \frac{dS}{dt}}{\rho \cdot c_p} - \frac{K_T \cdot A_T}{V \cdot \rho \cdot c_p} \left(T^0 - T^0_{ag} \right) = \frac{dT^0}{dt}$ - for the bioreactor's jacket: $\frac{F_{ag}}{V_{ag}} \left(T^0_{agi} - T^0_{ag} \right) + \frac{K_T A_T}{V_{ag} \cdot \rho_{ag} \cdot c_{pag}} \left(T^0 - T^0_{ag} \right) = \frac{dT^0_{ag}}{dt}$

Tables 2 and 3 present the list of the variables and parameters of the mathematical model.

Table 2. Variables and parameters of the kinetic model

X	Biomass concentration		$g \cdot L^{-1}$
S	Substrate concentration		$g \cdot L^{-1}$
P	Alcohol concentration		$g \cdot L^{-1}$
k	Kinetic constant		h^{-1}
A	Pre-exponential factor in Arrhenius' equation	148 (calculated using experimental data)	h^{-1}

E_a	Activation energy	21424 (calculated using experimental data)	$J\cdot mol^{-1}$
A_1	Pre-exponential factor in Arrhenius' equation	$9.5 *10^{8}$ [a]	h^{-1}
E_{a1}	Activation energy	55000[a]	$J\cdot mol^{-1}$
A_2	Pre-exponential factor in Arrhenius' equation	$2.55*10^{33}$	h^{-1}
E_{a2}	Activation energy	220000[a]	$J\cdot mol^{-1}$
R	Universal gas constant	8.31	$J\cdot mol^{-1}\cdot K^{-1}$
T^0	Temperature in bioreactor	291 and 301	K
K_s	Substrate limitation constant	0.2[a]	$g\cdot L^{-1}$
d	Pseudo-constant of the biomass	1.67 (calculated using experimental data)	
f	Pseudo-constant of the biomass	0.34	
α	Pseudo-order of the substrate	0.69[b]	
β	Pseudo-order of the alcohol	0.32[b]	
η	Efficiency in alcohol of fermentation reaction	48[b]	%
S_0	Steady-state operation point of substrate	180	$g\cdot L^{-1}$
P_0	Steady-state operation point of alcohol	0	$g\cdot L^{-1}$
t	Time		h
μ_{max}	Maximum specific growth rate		h^{-1}
K_P	Alcohol limitation constant	0.14[c]	$g\cdot L^{-1}$
q_{pmax}	Maximum specific alcohol production rate	1.02[c]	$g\cdot g^{-1}\cdot cells^{-1}\cdot h^{-1}$
K_{SP}	Constant in the substrate term for ethanol production	1.68[c]	$g\cdot L^{-1}$
K_{PP}	Constant of fermentation inhibition by ethanol	0.07[d]	$g\cdot L^{-1}$
Y_{XS}	Ratio of cell produced per glucose consumed for growth	0.607[d]	$g\cdot g^{-1}$
Y_{PS}	Ratio of ethanol produced per glucose consumed for fermentation	0.435[c]	$g\cdot g^{-1}$

[a] [21, 22, 23], [b][9], [c] [10], [d] [24]

Table 3. Parameters of the kinetic model

K_T	Heat transfer coefficient	$3.6.10^{5}$ [a]	$J\cdot m^{-2}\cdot K^{-1}\cdot h^{-1}$
A_T	Heat transfer area	0.8[b]	m^2
F_{ag}	Flow of cooling agent	0.01[b]	$m^3\cdot h^{-1}$
V_{ag}	Volume of the jacket	0.2[b]	m^3
V	Volume of the mass of reaction	1[b]	m^3
T^0_{agi}	Temperature of cooling agent entering to the jacket	278[b]	K
ΔH_r	Reaction heat of fermentation	-98465[c]	$J\cdot mol^{-1}$
ρ	Density of the mass of reaction	1100[b]	$kg\cdot m^{-3}$
ρ_{ag}	Density of cooling agent	999.8[a]	$kg\cdot m^{-3}$
c_p	Heat capacity of mass of reaction	3391[b]	$J\cdot kg^{-1}\cdot K^{-1}$
c_{pag}	Heat capacity of cooling agent	4217[a]	$J\cdot kg^{-1}\cdot K^{-1}$
T^0_{ag}	Temperature of cooling agent in the jacket		K

[a] [25], [b] experimental data, [c] [26]

The state vector is given by eq. (1)

$$\boldsymbol{x} = \begin{pmatrix} \boldsymbol{X} \\ \boldsymbol{S} \\ \boldsymbol{T}^0 \end{pmatrix} = \begin{pmatrix} \boldsymbol{x}_1 \\ \boldsymbol{x}_2 \\ \boldsymbol{x}_3 \end{pmatrix} \tag{1}$$

where **S** represents the substrate concentration, **X** - the biomass concentration and $\boldsymbol{T}^0$ - the temperature inside the bioreactor. The state vector of the process is extended with other two state variables: the concentration of the metabolism product (**P**), noted with $\boldsymbol{x_4}$ and the temperature of the cooling agent inside the jacket, $\boldsymbol{T^0_{ag}}$, noted with $\boldsymbol{x_5}$. It has to be mentioned that for the last two state variables the DSA method was not applied.

The flow of the cooling agent ($\boldsymbol{F_{ag}}$) is considered the control variable: $\boldsymbol{u}_1 = \boldsymbol{F}_{ag}$. The parameters of the mathematical model are the following: the pre-exponential factors in Arrhenius' equation (**A** and $\boldsymbol{A}_1$), the ratios between the activation energy and the universal gas constant ($\frac{\boldsymbol{E}_a}{\boldsymbol{R}}$ and $\frac{\boldsymbol{E}_{a1}}{\boldsymbol{R}}$) as kinetics characteristics and the heat transfer area ($\boldsymbol{A_T}$) as a design characteristic. The parameters' vector is as follows:

- for the exponential growing phase

$$\boldsymbol{p} = \begin{pmatrix} \boldsymbol{A}_1 \\ \dfrac{\boldsymbol{E}_{a1}}{\boldsymbol{R}} \\ \boldsymbol{A}_T \end{pmatrix} = \begin{pmatrix} \boldsymbol{p}_1 \\ \boldsymbol{p}_2 \\ \boldsymbol{p}_3 \end{pmatrix} \tag{2}$$

- for the decay phase

$$\boldsymbol{p} = \begin{pmatrix} \boldsymbol{A} \\ \dfrac{\boldsymbol{E}_{a}}{\boldsymbol{R}} \\ \boldsymbol{A}_T \end{pmatrix} = \begin{pmatrix} \boldsymbol{p}_1 \\ \boldsymbol{p}_2 \\ \boldsymbol{p}_3 \end{pmatrix} \tag{3}$$

Considering the notations from eqs. (1), (2) and (3) the mathematical model presented in Table 1 has been transformed, as shown in Table 4.

Table 4. The state space mathematical model of the alcoholic fermentation process

Current phase	**Equations**
Kinetic model	
Latent phase	$\boldsymbol{t} = \dfrac{\boldsymbol{a}}{\boldsymbol{x}_3} + \boldsymbol{b}$
Exponential growing phase	- biomass: $\mu_{\max} = \boldsymbol{p}_1 \cdot \boldsymbol{e}^{-\frac{p_2}{T^0}} - \boldsymbol{A}_2 \cdot \boldsymbol{e}^{-\frac{E_{a2}}{R \cdot T^0}}$

Current phase	**Equations**
	$\frac{dx_1}{dt} = \mu_{\max}\cdot\left(\frac{x_2}{K_S + x_2}\right)\cdot e^{-K_P\cdot x_4}\cdot x_1$ - alcohol: $\frac{dx_4}{dt} = q_{p\max}\cdot\left(\frac{x_2}{K_{SP} + x_2}\right)\cdot e^{-K_{PP}\cdot x_4}\cdot x_1$ - substrate: $\frac{dx_2}{dt} = -\left(\frac{1}{Y_{XS}}\cdot\frac{dx_1}{dt}\right) - \left(\frac{1}{Y_{PS}}\cdot\frac{dx_4}{dt}\right)$
Decay phase	- biomass: $\frac{dx_1}{dt} = f\cdot x_1\cdot p_1\cdot e^{-\frac{p_2}{x_3}}$ - alcohol: $\frac{dx_4}{dt} = -\eta\cdot\frac{dx_2}{dt}$ - substrate: $\frac{dx_2}{dt} = -p_1\cdot e^{-\frac{p_2}{x_3}}\cdot x_2^{\alpha}\cdot x_4^{\beta}$
Energetic model	
All phases	- for bioreactor: $\frac{dx_3}{dt} = 5.54\cdot\frac{\Delta H_r\cdot\frac{dx_2}{dt}}{\rho\cdot c_p} - \frac{K_T\cdot p_3}{V\cdot\rho\cdot c_p}\cdot\left(x_3 - x_5\right)$ - for the bioreactor's jacket: $\frac{dx_5}{dt} = \frac{u}{V_{ag}}\cdot\left(T_{agi}^{0} - x_5\right) + \frac{K_T\cdot p_3}{V_{ag}\cdot\rho_{ag}\cdot c_{pag}}\cdot\left(x_3 - x_5\right)$

The following initial conditions have been taken into consideration for the simulation of the mathematical model:

$$x_0 = \begin{pmatrix} x_{1,0} \\ x_{2,0} \\ x_{3,0} \end{pmatrix} = \begin{pmatrix} X_0 \\ S_0 \\ T_i^0 \end{pmatrix} \tag{4}$$

Direct sensitivity analysis (DSA)

The analysis of the dynamic system, based on the aforementioned mathematical model, was carried out using DSA. As it has been specified before, the main purpose of the DSA is the study of how the variation in the inputs of a model (biomass, substrate concentrations and temperature) can be apportioned, qualitatively or quantitatively, to different sources of variation.

Direct sensitivity analysis in relation with the initial state

The sensitivities' matrix in relation with the initial state, $S_{x_0}^{x}(t,t_0)$, is defined by the relation (5) as mentioned in [18]:

$$S_{x_0}^{x}(t,t_0)=\left\{\lim_{\Delta x_{j,0}\to 0}\frac{\Delta_{j,0}x_i(t)}{\Delta x_{j,0}}\right\}_{\substack{i=\overline{1,n}\\ j=\overline{1,n}}}=\left\{\frac{\partial x_i}{\partial x_{j,0}}\right\}_{\substack{i=\overline{1,n}\\ j=\overline{1,n}}} \qquad (5)$$

The eq. (5) is equivalent with eq. (6)

$$\Delta_{x_0}x(t)=S_{x_0}^{x}(t,t_0)\Delta x_0 \qquad (6)$$

The state vector's variation, $\Delta_{x_0}x(t)$ corresponding to a Δx_0 variation of the initial state vector, satisfies the differential equation:

$$\dot{\Delta}_{x_0}x(t)=f_x(x(t),p)\Delta_{x_0}x(t) \qquad (7)$$

where $f_x(x(t),p)$ is the Jacobean matrix and *p* – the parameters' vector (in this case they are constant). In eq. (6) it can be noticed that $S_{x_0}^{x}(t,t_0)$ is the transition matrix of the states. This matrix represents the solution of the sensitivity differential equation:

$$\dot{S}_{x_0}^{x}(t,t_0)=f_x(x(t),p)S_{x_0}^{x}(t,t_0) \qquad (8)$$

Table 5 presents the elements of the Jacobian matrix, ***f***$_x$, for the sensitivity analysis in relation with the initial state.

Table 5. Jacobian matrix for the sensitivity analysis in relation with the initial state

Current phase	**Equations**
Kinetic model	
Exponential phase	$f_{x_1,1}=\mu_{\max}\cdot\left(\frac{x_2}{K_S+x_2}\right)\cdot e^{-K_P\cdot x_4}$; $f_{x_1,2}=\mu_{\max}\cdot\left[\frac{K_S}{(K_S+x_2)^2}\right]\cdot e^{-K_P\cdot x_4}\cdot x_1$
	$f_{x_1,3}=\left(p_1\cdot p_2\cdot e^{-\frac{p_2}{x_3}}-A_2\cdot\frac{E_{a1}}{R}\cdot e^{-\frac{E_{a1}}{R\cdot x_3}}\right)\cdot x_1\cdot\frac{1}{x_3^2}\cdot\frac{x_2}{K_S+x_2}\cdot e^{-K_P\cdot x_4}$
	$f_{x_2,1}=-\frac{1}{Y_{XS}}\cdot\mu_{\max}\cdot\left(\frac{x_2}{K_S+x_2}\right)\cdot e^{-K_P\cdot x_4}-\frac{1}{Y_{PS}}\cdot q_{p\max}\cdot\left(\frac{x_2}{K_{PS}+x_2}\right)\cdot e^{-K_{PP}\cdot x_4}$

Current phase	Equations
	$f_{x_2,2} = -\frac{1}{Y_{XS}} \cdot \mu_{\max} \cdot \left[\frac{K_S}{(K_S + x_2)^2}\right] \cdot e^{-K_P \cdot x_4} \cdot x_1 - \frac{1}{Y_{PS}} \cdot q_{p\max} \cdot \left[\frac{K_{PS}}{(K_{PS} + x_2)^2}\right] \cdot e^{-K_{PP} \cdot x_4} \cdot x_1$ $f_{x_2,3} = 0$; $f_{x_3,1} = 5.54 \cdot \frac{\Delta H_r}{\rho \cdot c_p} \cdot f_{x_2,1}$; $f_{x_3,2} = 5.54 \cdot \frac{\Delta H_r}{\rho \cdot c_p} \cdot f_{x_2,2}$; $f_{x_3,3} = -\frac{K_T \cdot p_3}{V \cdot \rho \cdot c_p}$
Decay phase	$f_{x_1,1} = f \cdot p_1 \cdot e^{-\frac{p_2}{x_3}}$; $f_{x_1,2} = 0$; $f_{x_1,3} = f \cdot x_1 \cdot p_1 \cdot \frac{p_2}{x_3^2} \cdot e^{-\frac{p_2}{x_3}}$ $f_{x_2,1} = 0$; $f_{x_2,2} = -k \cdot \alpha \cdot x_2^{\alpha-1} \cdot x_4^{\beta}$; $f_{x_2,3} = -p_1 \cdot \frac{p_2}{x_3^2} \cdot e^{-\frac{p_2}{x_3}} \cdot x_2^{\alpha} \cdot x_4^{\beta}$; $f_{x_3,1} = 0$; $f_{x_3,2} = 5.54 \cdot \frac{\Delta H_r}{\rho \cdot c_p} \cdot f_{x_2,2}$; $f_{x_3,3} = 5.54 \cdot \frac{\Delta H_r}{\rho \cdot c_p} \cdot f_{x_2,3} - \frac{K_T \cdot p_3}{V \cdot \rho \cdot c_p}$

Direct sensitivity analysis in relation with the parameters

The sensitivities' matrix in relation with the parameters, $S_p^x(t,t_0)$, is defined by the eq. (9) as mentioned in [18]:

$$S_p^x(t,t_0) = \left\{\lim_{\Delta p_j \to 0} \frac{\Delta_{p_j} x_i(t)}{\Delta p_j}\right\}_{\substack{i=\overline{1,n}\\ j=1,n}} = \left\{\frac{\partial x_i}{\partial p_j}\right\}_{\substack{i=\overline{1,n}\\ j=1,n}} \tag{9}$$

It is equivalent with eq. (10)

$$\Delta_p x(t) = S_p^x(t,t_0) \Delta p \tag{10}$$

The sensitivity's differential equation is the following:

$$\dot{S}_p^x(t,t_0) = f_p(x(t),p) S_p^x(t,t_0) \tag{11}$$

Table 6 presents the elements of the Jacobian matrix, ***f*$_x$**, for the sensitivity analysis in relation with the model parameters.

Table 6. The equations of the Jacobean matrix, ***f*x**, for the sensitivity analysis of the process state in relation with the time-invariant parameters

Current phase	Equations
Kinetic model	
Exponential phase	$f_{x_1,1} = e^{-\frac{p_2}{x_3}} \cdot \frac{x_2}{K_S + x_2} \cdot e^{-K_P \cdot x_4} \cdot x_1 \; f_{x_1,2} = -p_1 \cdot \frac{x_1}{x_3} \cdot e^{-\frac{p_2}{x_3}} \cdot \frac{x_2}{K_S + x_2} \cdot e^{-K_P \cdot x_4}$; $f_{x_1,3} = 0$; $f_{x_2,1} = 0$; $f_{x_2,2} = 0$; $f_{x_2,3} = 0$; $f_{x_3,1} = 0 \; f_{x_3,2} = 0$; $f_{x_3,3} = -\frac{K_T}{V \cdot \rho \cdot c_p} \cdot (x_3 - x_5)$
Decay phase	$f_{x_1,1} = f \cdot x_1 \cdot e^{-\frac{p_2}{x_3}}$; $f_{x_1,2} = -f \cdot x_1 \cdot \frac{p_1}{x_3} \cdot e^{-\frac{p_2}{x_3}}$; $f_{x_1,3} = 0$ $f_{x_2,1} = -e^{-\frac{p_2}{x_3}} \cdot x_2^{\alpha} \cdot x_4^{\beta}$; $f_{x_2,2} = -\frac{p_1}{x_3} \cdot e^{-\frac{p_2}{x_3}} \cdot x_2^{\alpha} \cdot x_4^{\beta}$; $f_{x_2,3} = 0$; $f_{x_3,1} = 5.54 \cdot \frac{\Delta H_r}{\rho \cdot c_p} \cdot f_{x_2,1}$; $f_{x_3,2} = 5.54 \cdot \frac{\Delta H_r}{\rho \cdot c_p} \cdot f_{x_2,2}$; $f_{x_3,3} = -\frac{K_T}{V \cdot \rho \cdot c_p} \cdot (x_3 - x_5)$

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