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STATISTICAL PROCESSING AND DYNAMIC MODELING OF AN ALCOHOLIC FERMENTATION PROCESS

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ABSTRACT. The experimental data obtained in this research work were used in combination with the response surface planning method, modeling and simulation to determinate the variables with major influence on the length of an alcoholic fermentation process. Then, based on the periods according to yeast cells physiological phases a non-linear, dynamic mathematical model for the same process was developed. This model involves equations corresponding to physiological phases of yeast cells, heat transfer equations and the dependence of kinetic parameters on temperature.

Keywords: *alcoholic fermentation process, response surface method, dynamic model*

INTRODUCTION

The alcoholic fermentation of white wine process is characterized for its complex dynamic response. The alcoholic fermentation in food industry have particular characteristics include the following: batch fermentation on natural complex media, anaerobic conditions due to CO_2 production, the composition of the grape juice, the, low media pH, the levels of the sulphur dioxide, the inoculation with selected yeasts and the interaction with other microorganisms. The models developed for this process consequently include the substrate inhibition of: microbial growth, substrate sugars and ethanol concentrations [1, 2, 3]. Many of these models offered good prediction of the important process variables, but most deviate significantly from the final ethanol concentration [4, 5]. The dead time, time delay in the instrument measurements, presence of variables that vary with time and high

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nonlinearities involving the variables are some examples that confirm the below affirmation [1]. For these reasons, modeling, simulation, and control of this system is still not a totally resolved problem.

In this article the author presents data base obtained on an alcoholic fermentation process that was made using different initial concentrations in yeast and sugar and also different temperatures in process control. All these data were processing by statistical models.

Statistical models are obtained by correlating experimental data [6]. Designing an empirical model does not require detailed representation of the mechanism of the real process; this also gives empirical models simplicity and consequently great versatility which sometimes is desirable.

In general, in designing a statistical model there must be taken the following steps [7]: determine the model structure; organizing and conducting experiments in the real process; interpreting and processing the results; deduction of the final form of the model equations and the calculation of the model coefficients and finally check of the validity of the model by analyzing the dispersion.

All these steps were made using the response surface planning method. The response surface design was used in a simulation study to determine the variables with major influence on the length of fermentation process. The variables selected to be observed in this study were: temperature, substrate concentration and yeast concentration.

The study was continued with a non-linear, dynamic mathematical model developed in periods, according to yeast cells physiological phases. The model presented, besides the detailed kinetic model, involves equations corresponding to physiological phases of yeast cells, heat transfer equations and the dependence of kinetic parameters on temperature. As a distinct modeling principle of each phase, the evolution curve of biomass in time, afferent to viable cells was used. The model was validated with the experimental obtained data.

This proposed mathematical model is taking part from an author's study concerning the estimation of state and parameters of fermentation process and the process control by expert system.

RESULTS AND DISCUSSION

Results for statistical model simulation

Using functions included in *Statistics Toolbox* from MATLAB computational software different second-degree polynomial empirical models were tested. The best fitted model was of the form:

$$t = c_0 + c_1 T^0 + c_2 X + c_3 T^0 S + c_4 X^2 \tag{1}$$

The coefficients c_0, …, c_4 of the polynomial model (1) were calculated using MATLAB function *regress* and the obtained equation is:

$$t = 1066.864 - 2.6196\,T^0 + 13.3294\,X - 0.0036\,T^0 S - 0.6452\,X^2 \quad (2)$$

The residual values calculated weren't high. The difference between experimental and estimated by the model presented in eq. (2) values was less than 3 % as it can be seen in Table 1.

Table 1. Comparison between experimental and model data

Experiment	Duration of the fermentation process, *t*			
	Measured, [h]	Calculated, [h]	Difference	
			[h]	[%]
1	76	76.67	0.67	0.87
2	49	49.67	0.67	1.34
3	95	95.67	0.67	0.70
4	76	74.00	-2	-2.70
5	101	101.00	0	0
6	59	59.00	0	0

The response surface modelling window (opened by the *rstool* function from MATLAB) constructed on the statistical equation is presented in Figure 1. This interactive interface is part of the *Statistics Toolbox* included in the MATLAB computing software environment where a sequence of plots are displayed, each showing a contour of the response surface against a single parameter, with all other parameters held fixed.

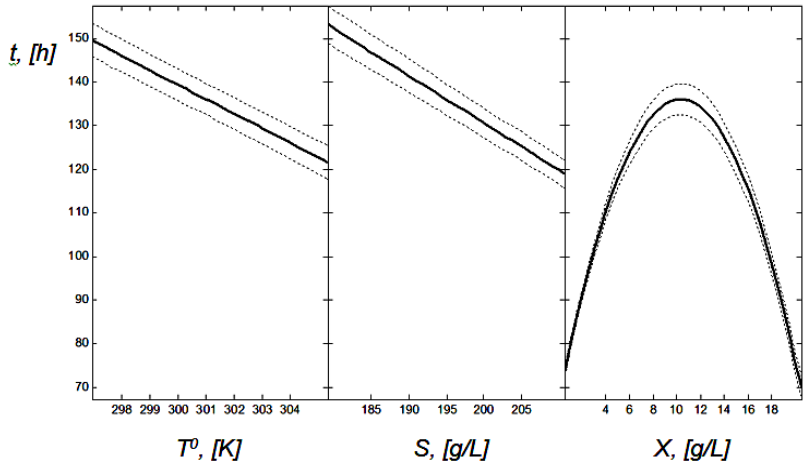

Figure 1. The *rstool* window of the statistical model where with solid lines are representing the response of the model and with dotted lines the interval for a global confidence of 0.95

From these graphical representations it can be observed the form of the response surface model obtained.

Studying this statistical equation it can be observed the followings:

1. The high value of free term shows that other factors also exist that influence the fermentation process. These factors can be: nutrients content, vitamins content of the grapes must etc.
2. The temperature influence on the fermentation process is emphasized by the high value of its coefficient that corresponds to the reality.
3. The value of *X* coefficient demonstrates that the strain of selected yeast used in fermentation is very important. The sort of yeast can influence the fermentation rate, the duration of the process and so the final alcohol and aroma concentrations.
4. The combined action of the temperature and substrate concentration (the sugars fermentescible content) is weak but in negative sense. This observation confirms the fact that a great content in substrate leads to a slow fermentation (a high concentration in substrate can be an inhibitor for the yeast development). Also, a weaker content in sugar can lead to a very short fermentation with a low concentration in alcohol at the end, without aromas and with a high rate of CO_2 production for a short period.

Results for dynamic mathematical model simulation

Batch fermentation nonlinear mathematical model has the following aspects: an equation for latent phase of fermentation that describes the phase period of the dependence with temperature; the Aiba model for exponential growing phase and Bovée-Strehaiano model for decay phase with two equations: one for substrate consumption and the other for alcohol formation; an equation that describes the biomass behavior along the fermentation, different for the Phase 2 and for the Phase 3, respectively; and an energy balance model in which the rate of change of mass of reaction temperature (dT^0/dt) is a result of the balance between the rate of heat generation due to fermentation and the rate of heat transfer to the cooling medium from the bioreactor jacket. The Aiba model was chosen because it introduces the ethanol inhibitory effect and Bovée-Strehaiano model has accurately describes for the substrate consumption and the evolution of the alcohol concentration in the decay phase.

For latent phase a linear equation was obtained, describing the dependence of the phase period on temperature. The equation is valid for a period between 0–100 h, a period confirmed by the data in the literature [8].

Figure 2 presents the fitting curve calculated by the polynomial regression method.

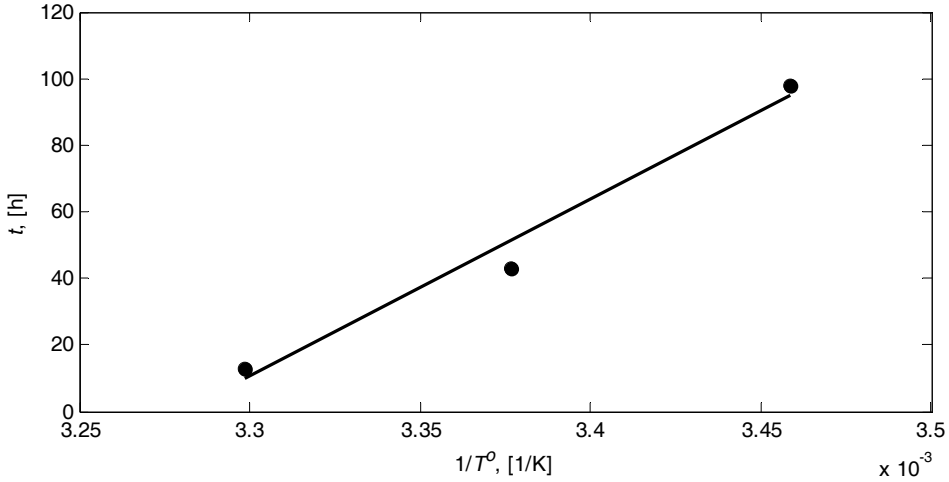

Figure 2. The tendency curve for latent phase, calculated based on experimental data

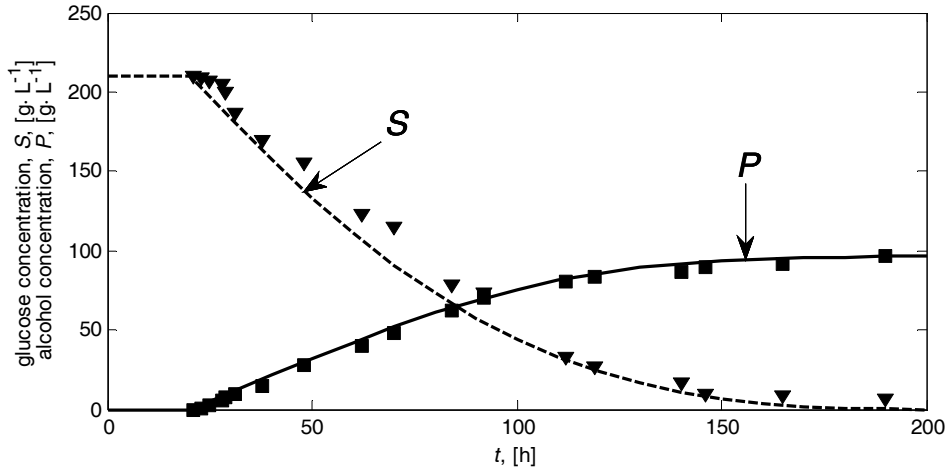

Figure 3. Authors' model: The evolution of glucose and alcohol versus time, comparison between experimental values (▼ glucose, ■ alcohol) and simulation results (lines)

The simulation results considering this model are presented in Figures 3, 4 and 5, for 210 g·L^{-1} substrate initial concentration and 301 K fermentation temperature.

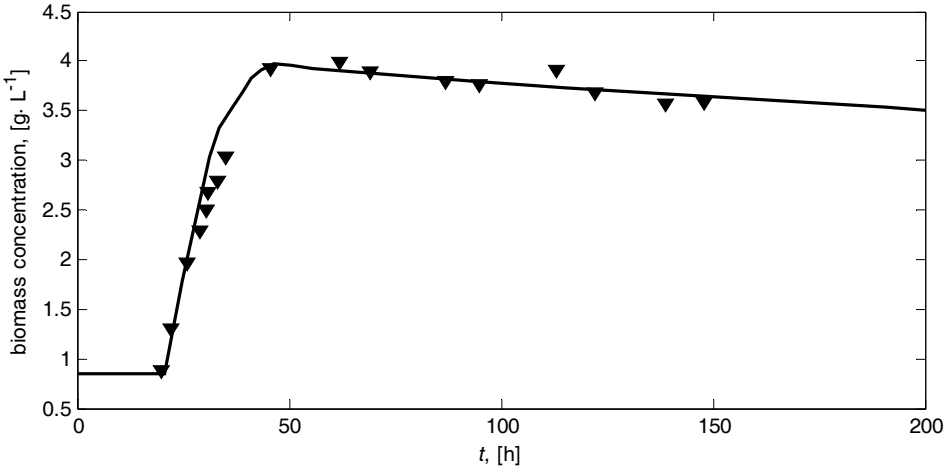

Figure 4. Authors' model: Comparison of biomass simulation results (line) with experimental ones (▼)

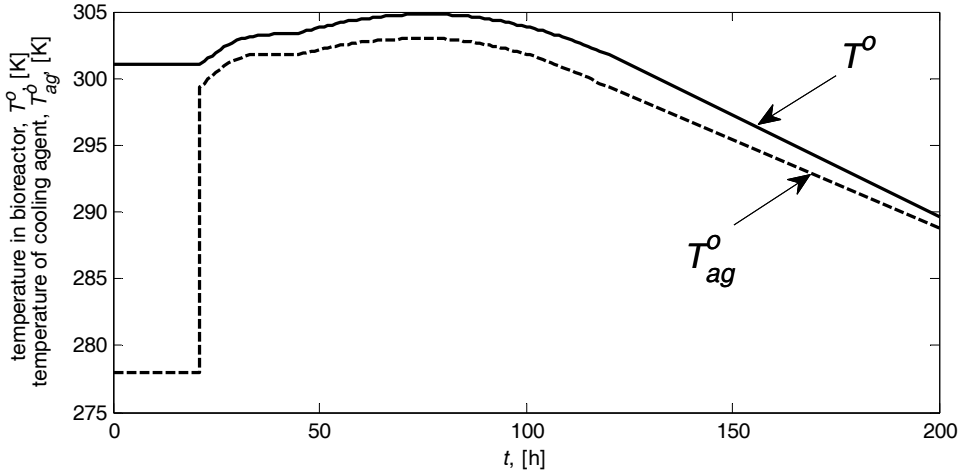

Figure 5. The temperatures of fermentation medium and cooling agent simulation

The maximal difference between experimental and simulation results are less than 10 %. The model has been tested for a grape must with an initial concentration in substrate varying between 180 and 210 g·L^{-1}, a fermentation temperature between 299 and 303 K and without aeration.

CONCLUSIONS

In the present article the authors proposed a statistical model for the classic alcoholic fermentation process of the white wine by correlating the experimental data. This model emphasizes the influence of some variables to the fermentation process length (temperature, initial substrate concentration and initial biomass concentration). The interpretation of the simulation results was made by using the response surface planning method. Then, based on these observations a dynamic mathematical model was developed and validated. The system is described by a set of non-linear equations corresponding to the physiological phases of yeast cells: substrate, biomass and alcohol dynamic behaviour, heat transfer equations and the dependence of kinetic parameters on temperature. The results obtained in this research work permitted to the authors to continue with the estimation of the singular and in group influences of input variables – substrate and biomass – on the fermentation process and to design the suitable control technique.

EXPERIMENTAL

The alcoholic fermentation process of the white wines has been studied in the research laboratory of Modeling, simulation and advanced control of the technological processes and bio-processes from food industry, "Lucian Blaga" University of Sibiu, the Faculty of Agricultural Sciences, Food Industry and Environmental Protection.

In the first part, the research experiments have been focused on the influence of some variables to the fermentation process length, t. The selected variables were: temperature, T^0; substrate concentration, S and biomass concentration, X. The medium used for inoculums and culture contained: *Saccharomyces cerevisiae* yeast wine YEPD, 5 $g \cdot L^{-1}$ KH_2PO_4, 2 $g \cdot L^{-1}$ $(NH_4)_2SO_4$, 0.4 $g \cdot L^{-1}$ $(MgSO_4)$ x $7H_2O$ and fermentation medium formed by malt extract. The content in sugar of malt extract was matched with saccharose, if was necessary. A EVO continuously stirred bioreactor equipped with pH, temperature, level and stirred speed control, disolved O_2, emitted CO_2 and O_2 sensors and analysers was used [9].

Fermentation parameters measurements

The cells concentration has been calculated on the base of three different parameters: optical density, dry substance and total cells number (Thoma room method). The glucose has been measured by DNS method and the alcohol by high performance liquid chromatography (HPLC).

The operating values of the variables are presented in Table 2.

Table 2. The operating values of the considerating variables and the periods of fermentation processes

Experiment' number	Temperature, T^0 [K]	Initial substrate concentration, S_0 [g·L^{-1}]	Initial biomass concentration, X_0 [g·L^{-1}]	Duration of fermentation process, t [h]
1	297	210	1	76
2	305	210	1	49
3	301	180	1	95
4	301	200	1	76
5	301	210	5	101
6	301	210	20	59

These experimental data were used by the response surface methodology to obtain an empirical model of the process to correlate the response of the system, the duration of the fermentation process with the considered parameters of the process: temperature; initial substrate concentration and initial biomass concentration. Necessary calculation were performed using MATLAB computing software.

In the second part, the research work was continued with the experiments necessary to develop a non-linear, dynamic mathematical model for the alcoholic fermentation process.

The operating variables and parameters for the experimental plant were as follows: working volume 8 L; temperature: 291 K and 301 K; stirring speed 150 rpm.; pH 3.8 and influent glucose concentrations: 180 g·L^{-1} and 210 g·L^{-1}.

Without aeration, the necessary oxygen was that dissolved in malt extract.

In the dynamic mathematical model, as a distinct modeling principle of each phase, the evolution curve of biomass over the time for the viable cells has been used. The evolution of yeast population curve has been divided in correlation with phenomenological aspects of the development of microorganisms as follows: latent phase, growing phase and decay phase.

The equations of this model are presented in Table 3.

The parameters of the model were adjusted by means of non-linear programming methods, which compare model predictions with experimental data and minimize the errors. The variables and parameters of the model are presented in Tables 4 and 5.

Also, in MATLAB simulation system the numerical simultaneous integration of the model equations has been done.

Table 3. The equations of the proposed model

Phase		Equations
	Kinetic model	
Latent phase [10]		$t_{lat} = \frac{a}{T^0} + b$; a = 558,002 and b = 1,833.2
Exponential growing phase [11]	- 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:	$\frac{dP}{dt} = q_{pmax} \cdot \left(\frac{S}{K_{SP} + S}\right) \cdot e^{-K_{pp} \cdot P} \cdot X$
	- 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 [10,12]	- 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 [10]	- for 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 bioreactor's jacket:	$\frac{F_{ag}}{V_{ag}}\left(T^0_{agi} - T^0_{ag}\right) + \frac{K_T A_T}{V_{ag} \cdot \rho_{ag} \cdot c_{pag}}\left(T^0 - T^0_{ag}\right) = \frac{dT^0_{ag}}{dt}$

Table 4. 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	21,424 (calculated using experimental data)	$J \cdot mol^{-1}$
A_1	Pre-exponential factor in Arrhenius' equation	$9.5 \cdot 10^{8}$ [a]	h^{-1}
E_{a1}	Activation energy	55,000[a]	$J \cdot mol^{-1}$
A_2	Pre-exponential factor in Arrhenius' equation	$2.55 \cdot 10^{33}$	h^{-1}
E_{a2}	Activation energy	220,000[a]	$J \cdot mol^{-1}$
R	Universal gas constant	8.31	$J \cdot mol^{-1}K^{-1}$
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]	$g \cdot g\ cells^{-1}h^{-1}$
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][4, 13, 14];
[b][12]; [c][15];
[d][16]

Table 5. Parameters of the kinetic model

K_T	Heat transfer coefficient	$3.6 \cdot 10^{5}$ [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	0.002[b]	L
V	Volume of the mass of reaction	1[b]	L
T^0_{agi}	Temperature of cooling agent at jacket inlet	278[b]	K
ΔH_r	Reaction heat of fermentation	–98,465[c]	$J \cdot mol^{-1}$
ρ	Density of the mass of reaction	1,100[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	3,391[b]	$J \cdot kg^{-1} \cdot K^{-1}$
c_{pag}	Heat capacity of cooling agent	4,217[a]	$J \cdot kg^{-1} \cdot K^{-1}$
T^0_{ag}	Temperature of cooling agent in the jacket		K

[a][17]; [b]experimental data; [c][18]

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