NON-ISOTHERMAL MODEL OF THE YEASTS GROWTH IN ALCOHOLIC FERMENTATIONS FOR HIGH QUALITY WINES

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ABSTRACT
Since, one of the most critical variables influencing the bioprocess in winemaking is temperature; a non-isothermal phenomenological model for yeast kinetics in winemaking fermentation was developed. The proposed model, based on a previous published by authors, considers a new expression for the maximum specific cellular growth rate and two more kinetic parameters depending on operation temperature. They are: specific cellular death rate and the carbon dioxide released at 85-95% of its maximum value. The developed model was validated by accurately predicting the cellular growth of own lab-scale fermentations and, it also verifies to follow the typical trend of literature experimental data. For such purpose, model performance between 10 to 40ºC was evaluated via simulations for constant and variable temperature predefined trajectories. Since obtained results are satisfactory, this model can be used to track complex temperature profiles to achieve high quality wines, as well as, in other control and optimization strategies.

Keywords: non isothermal operation, wine fermentation, phenomenological modelling, yeasts growth

1. INTRODUCTION

Argentina is the largest wine producer in South America. A range of fine wines has raised their incorporation in the most important international markets in the last years, and some of them are among the top rated wines in the world. The customers' increasing demand for high quality wines and its marked preferences for outstanding organoleptic properties of wine, presents new challenges for the winemaking technology.

The bioreactor bulk temperature is a well-known critical variable that determine the kinetics of the fermentation (Coleman, Fish, and Block 2007).
Temperature directly influences on microbial ecology of grape must and the biochemical reactions of yeasts (Fleet and Heard 1993). Moreover, it is known that *Saccharomyces cerevisiae* synthesizes aroma compounds during the winemaking fermentations. It is also stated that the production, quality, quantity and rate of yeast-derived aroma compounds is affected by the temperature used. Typically, temperatures ranging between 15ºC (for white wines) and 30ºC (for red wines) are used. Furthermore, most winemaking fermentations are not carried out at constant temperature. Experiments conducted at constant temperature, revealed that production of compounds related to fresh and fruity aromas is favoured at temperatures near 15°C, while flowery related aroma compounds are better produced at 28°C (Molina, Swiegers, Varela, Pretorius, and Agosin 2007).

In relation with some sensory-relevant flavour generation, it was suggested that higher temperatures, near 28 ºC, are only beneficial at the start of fermentation, and then lower temperatures will be advantageous due to the decrease of the volatility and removal of the aroma compounds formed (Fischer 2007).

It is evident that temperature strongly affects the quality of wine (Torija, Rozès, Poblet, Guillamón, and Mas 2003), and new technologies must include variable temperature trajectories throughout the fermentation.

The development of efficient control strategies for the main operation variables in fermentations such as pH, temperature, dissolved oxygen concentration; agitation speed, foam level, and others need accurate dynamic models (Morari and Zafiriou 1997, Henson 2003). Also, wine fermentation models are useful tools to assure wine quality and reproducibility among batches (Zenteno, Pérez-Correa, Gelmi, and Agosin 2010).

In previous reports, the authors have developed isothermal and non-isothermal first-principles and hybrid neural models, and an improved isothermal phenomenological model with satisfactory capability to approximate the wine fermentation profiles (Vallejo, Aballay, Toro, Vazquez, Suarez, and Ortiz 2005; Ortiz, Aballay, and Vallejo 2006; Aballay, Scaglia, Vallejo, and Ortiz 2008; Scaglia, Aballay, Mengual, Vallejo, and Ortiz 2009).

The objective of this work is to propose a non-isothermal phenomenological model for wine fermentation kinetics, able to predict with enough accuracy yeasts growth and track complex temperature profiles from 10 to 40ºC, to produce wines with high quality.

The proposed model couples mass and energy balances predicting the behaviour of the main state process variables: viable cells, substrate (total fermentable sugars) and ethanol concentrations, carbon dioxide released, and the bioreactor temperature. It is based on the one developed by Scaglia, Aballay, Mengual, Vallejo, and Ortiz (2009), that possesses a good performance for isothermal fermentations, and the one presented by Aballay, Scaglia, Vallejo, and Ortiz (2008) for non-isothermal fermentations. In the latter case, operating temperature ranges from 20 to 30ºC.

A set of ordinary differential equations (ODE), including the heat transferred between the reactor and its cooling jacket, constitute the present model. Balances have been coupled by means of the Arrhenius equation which describes the temperature influence on the cell growth (Aballay, Vallejo, and Ortiz 2006; Aballay, Scaglia, Vallejo, and Ortiz 2008) and death rates (Phisalaphong, Srirattana, and Tanthapanichakoon 2006).

Kinetic parameters of the model were adjusted using experimental data obtained from anaerobic lab-scale cultures of *Saccharomyces cerevisiae* (killer), and/or *Candida cantarelli* yeasts in Syrah must (red-grape juice), see Toro and Vazquez (2002). In the case of the specific parameters in Arrhenius expression, they were adjusted by the least-square method.

Since in practice, the temperature in the bioreactor must be maintained constant at a certain level to avoid the quality product decrease, or varied tracking a predefined trajectory to achieve a wine with particular organoleptic properties (Ortiz, Vallejo, Scaglia, Mengual, and Aballay 2009), the performance of model was tested via simulation to validate it. Results from model simulations and validation are shown. They state suitable agreement with own experimental and published data, predicting fermentation evolution without significant retards. The latter allows model application in advanced control strategies for the winemaking process.

The work is organised as follows. First, the lab-scale fermentation experiments carried out with variable temperature to validate the model to develop are described. Second, the non-isothermal kinetic modelling of the bioprocess from formerly developed isothermal and non-isothermal models is presented. Third, model simulation results are compared to: literature data to verify they well track cellular growth trends and, own experimental data for its validation. Fourth, a discussion on the appliance of the obtained model in complex control and optimization schemes in winemaking, and conclusions are exposed.

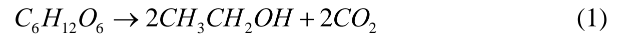
2. MATERIALS AND METHODS

Microorganism: *Saccharomyces cerevisiae*, (strain PM16, obtained in our laboratory[(b)]), maintained in agar-YEPD (yeast extract-peptone-dextrose), and propagated in red-grape must. Culture medium: concentrated red-grape must, properly diluted to obtain 23ºBrix at 23ºC, initial pH was set to 3.5, and sterilized at 121ºC during 20 minutes. Fermentations (FER3): 250 mL flasks containing 100 mL of sterile must was inoculated with $2x10^6$ yeast, capped with Muller's valves, and cultured in anaerobic conditions, at temperature following the sequence from 23ºC to 18ºC, presented in Fig. 4. Samples were taken each 6 hours during the first 7 days and then each day; yeasts were accounted by means a Neubauer chamber, the fermented must was centrifugated and the supernatant was maintained for analytical determinations.

3. MATHEMATICAL MODEL

In winemaking conditions, the main bio-reactions can be synthesised by the reductive pathway S ➔ X + P + CO_2, this reaction means that substrates (S, glucose and fructose and sucrose, after their hydrolysis as the limiting substrate), in anaerobic conditions, are metabolised to produce a yeast population (X), ethanol (P, mainly produced by yeast through the Embden-Meyerhof-Parnas metabolic pathway) and carbon dioxide (CO_2).

The ethanol-formation reaction from glucose is:



$$C_6H_{12}O_6 \rightarrow 2CH_3CH_2OH + 2CO_2 \quad (1)$$

The metabolite accumulation in the extra-cellular medium has been modelled by a set of ODE based on mass balances on X, S, P and CO2 which change with time *t* [h] like in the isothermal model of Scaglia, Aballay, Mengual, Vallejo, and Ortiz (2009), which can be seen for further details, and it is summarised as Eqs. 2 through 5:

Viable cells:

$$\frac{dX}{dt} = \frac{e^{-(CO_2 - C0_{2(95)})}}{e^{(CO_2 - C0_{2(95)})} + e^{-(CO_2 - C0_{2(95)})}} A\mu_{\mathrm{m}} \frac{S}{S + Ks\,B^a} \ldots$$
$$\ldots X\left(1 - \frac{X}{A\mu_{\mathrm{m}} \frac{S}{(S + Ks\,B^a)\beta}}\right) + \left[1 - \frac{e^{-(CO_2 - C0_{2(95)})}}{e^{(CO_2 - C0_{2(95)})} + e^{-(CO_2 - C0_{2(95)})}}\right]\left(CX\frac{dS}{dt} - K_d X\right) \quad (2)$$

Substrate:

$$\frac{dS}{dt} = \frac{1}{Y_{X/S}}\left[-X\left(\mu_{\mathrm{m}} \frac{S}{S + Ks\,B^b} - E\mathrm{X}\right)\right] - \mathrm{FX} \quad (3)$$

Carbon dioxide released:

$$\frac{dCO_2}{dt} = G\mu_m \frac{S}{S + Ks\,B^c} X + \\ + \frac{d}{dt}\left(H\mu_m \frac{S^2}{\left(S + Ks\,B^d\right)\left(S + Ks\,B^e\right)} X + I\,X \right) \quad (4)$$

Ethanol:

$$\frac{dP}{dt} = \frac{1}{Y_{CO_2/P}} \frac{dCO_2}{dt} \quad (5)$$

Numerical values of preceding model parameters and their description are shown in Table 1 in the Appendix.

Model assumptions are: other mass balance parameters of the model, including pH, are constant. Fermentation is not nitrogen source-limited; this is viable, based on information about the chemical composition of the local red-grape musts. Moreover, local winemakers only add nitrogen supplementation, in excess, to correct the white-grape musts. In the energy balance (Eq. 6): heat losses due to CO_2 evolution, water evaporation and ethanol and flavour losses are neglected; the average grape juice-wine density and specific heat, and all physical properties are uniform in the fermenting mass bulk. They are constant with the (bioreactor) temperature T [K] and time. Convective heat transfer coefficient of fermentation mass, implicitly included in Eq. (6), is constant (Colombié, Malherbe, and Sablayrolles 2007). In the cooling jacket: water properties variations and the fouling factor in jacket side are neglected. Heat transfers by radiation and conduction are negligible.

The non-isothermal kinetic model is constituted by mass balances of the before-mentioned model and the energy balance in the reactor and its cooling water jacket.

$$\frac{d\left(\rho_r \cdot V_r \cdot Cp_r \cdot T\right)}{dt} = Y_{H/CO_2} \cdot V_r \cdot \frac{dCO_2}{dt} - Q \quad (6)$$

V_r [m^3] is the volume. $Y_{H/CO2}$ [W·h produced/kg CO_2 released] is the energy due to the carbon dioxide released by the bio-reaction. It was obtained by stoichiometry (Eq. 1) from $Y_{H/S}$, the likely energetic yield on substrate consumed. Q [W] represents the exchanged heat between the fermenting mass and the cooling jacket (see details in Aballay, Scaglia, Vallejo, and Ortiz 2008). ρ_r [kg m^{-3}] and C_{pr} [W·h kg^{-1} K^{-1}] are density and specific heat of the fermenting mass.

Mass and energy balances are coupled by means of: Arrhenius' equation for maximum specific cellular growth and death rates, μ_m [h^{-1}] and K_d [h^{-1}] respectively, and polynomial regressions for dimensionless coefficients L within μ_m, and M within the parameter for estimation of the carbon dioxide released at 85-95% of its maximum value $CO_{2\,(95)}$. The above mentioned bioprocess variables progress in time and, temperature influence on them and their parameters can be expressed in a general way as: $\{dX/dt,\, dS/dt,\, dCO_2/dt \text{ and } dP/dt\} = ...$
$...f(X, S, CO_2, \mu_m(T), K_d(T), CO_{2(95)}(T))$.

The mathematical expressions for the three kinetic temperature-dependent parameters are given in Eqs. (7), (8) and (9):

$$\mu_m = \gamma.L.\frac{T \cdot e^{-\frac{E_a}{R \cdot T}}}{1 + e^{-\frac{\Delta G_d}{R \cdot T}}} \quad (7)$$

γ is the maximum cellular growth rate per Kelvin degree [h^{-1}K^{-1}], L is a dimensionless coefficient depending on the temperature (Eq. 10), E_a is the activation energy for cell growth [kJ kmol^{-1}] and ΔG_d [kJ kmol^{-1}] is Gibbs free energy change of the fermentation reaction. R is general gases constant [kJ kmol^{-1} K^{-1}].

$$K_d = \begin{cases} K_{d,0} \cdot T \cdot e^{\frac{-E_d}{R \cdot T}} \; if\ T \le 304K \\ 0.0165\ Otherwise \end{cases} \quad (8)$$

K_d replaces parameter D in the model of Scaglia Aballay, Mengual, Vallejo, and Ortiz (2009). $K_{d,0}$ is the specific cellular death rate per Kelvin degree and E_d is the activation energy for cellular death [kJ kmol^{-1}].

Moreover, parameters E_a, ΔG_d, $K_{d,0}$, and E_d, were adjusted by the least-square method, using experimental data obtained from anaerobic lab-scale cultures of *Saccharomyces cerevisiae* (killer) and *Candida cantarelli* yeasts, with Syrah must in batch mode (Toro and Vazquez, 2002).

$$CO_{2(95)} = CO^*_{2(95)} \cdot M \quad (9)$$

$CO^*_{2\,(95)}$ is a carbon dioxide value, chosen between the 85% and 95% of the total carbon dioxide released at constant temperature (296K) and, M is a dimensionless coefficient depending on the temperature (Eq. 11).

$$L = f\,T^5 - g\,T^4 + h\,T^3 - i\,T^2 + j\,T - k \quad (10)$$

$$M = l\,T^5 - m\,T^4 + n\,T^3 - o\,T^2 + p\,T - q \quad (11)$$

Where f, g, h, i, j, k, l, m, n, o, p, and q are own coefficients of the model, see Table 2 in the Appendix.

Initial conditions and another parameters of the model used for simulating experimental fermentations from literature are described in Table 2 in the Appendix. Those fermentations are mentioned as: FERT, of Torija Rozès, Poblet, Guillamón, and Mas (2003), FER1 and FER2 (Toro and Vazquez 2002) and FER3 from own data. The latter was carried out to validate the present model. In addition, maximum values of viable cells concentration achieved during the fermentations are included in Table 2 (Appendix) as well.

4. SIMULATIONS

4.1. Results

The developed model was tested via simulations in similar conditions than experimental fermentations from literature. To carry out the simulations, the model was codified in Matlab™ software. Results are presented in this section.

Results are expressed on normalised yeast concentrations in order to allow comparisons between different yeast strains, having different masses.

Figure 1, represents the yeasts growth profiles attained by simulations at different constant temperatures ranging from 283K (10ºC) to 313K (40ºC) for the same initial conditions of substrate and yeasts concentration. It can be seen that yeast growth and the maximum cells concentration are favoured at temperatures between 290 and 300K. At lower temperatures than 290K, it is observed that the yeast growth is delayed and the maximum cells concentration achieved is lower than attained between 290 and 300K. For initial temperatures higher than 300K, not only the yeast growth is diminished but also the yeast death is anticipated. This situation may be due to the dual effect of temperature over the optimal growth conditions and the ethanol-tolerance.

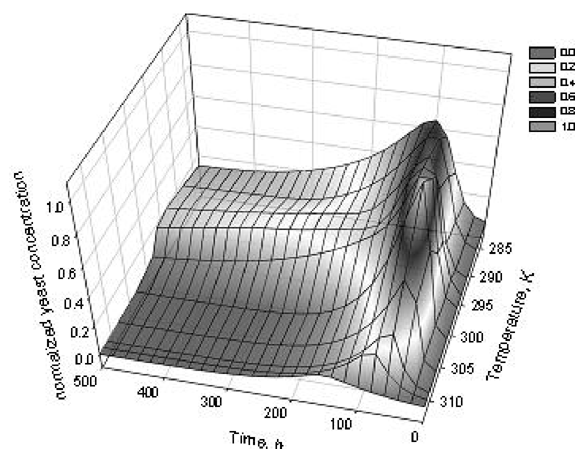


Figure 1: Normalised Viable Cells Profiles: Modelled Results at Different Temperatures (283-313K); Max. = $109.74 \cdot 10^6$ cfu mL^{-1} (Parameter Values from FER2)

In order to contrast the simulation results obtained with experimental data, it was constructed a 3D-mesh plot (Fig. 2), taking data from literature and reporting experiences of wine fermentations at different constant initial temperatures (Torija, Rozès, Poblet, Guillamón, and Mas 2003).

Comparing Figs. 1 and 2, it can be seen that the behaviour of the model approximates appropriately to the mentioned experimental data from literature. Even though, in the last case (Fig. 2), at low temperatures, the yeast growth seems to be more retarded than in Fig. 1. It is necessary to point out that yeast used by Torija, Rozès, Poblet, Guillamón, and Mas (2003), was not the same used to fit the model in Fig. 1. Thus, these yeasts may have similar performance but not exactly the same, because each strain has a proper behaviour pattern. Although, the comparison between Figs. 1 and 2 is promising, the model performance must be contrasted with experiences carried out at variable temperature profiles.

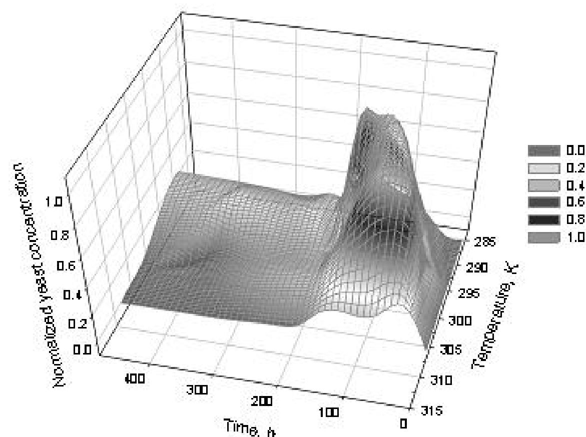
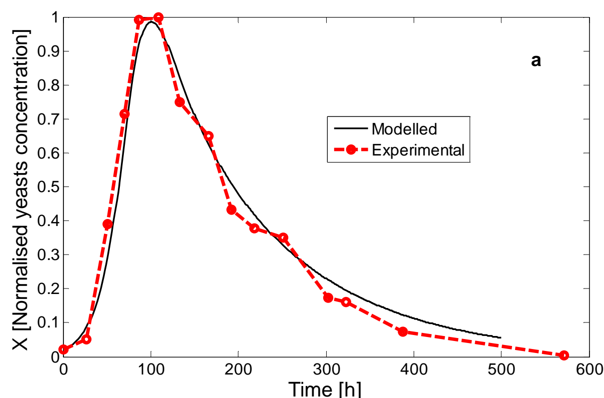


Figure 2: Normalised Viable Cells Profiles: Experimental Fermentations at Different Temperatures (288-308K) (Torija, Rozès, Poblet, Guillamón, and Mas 2003); Max.= $195 \cdot 10^6$cfu mL^{-1}

4.2. Model validation

The model validation was accomplished by simulation as well, using initial conditions of different own lab-scale experimental data sets at different constant and variable temperature profiles.

As Fig. 3 shows that for fermentations FER1 and 2 (both at constant 296±1K), the model proposed has an acceptable prediction with only up to 10 hours average in retard regarding experimental data.



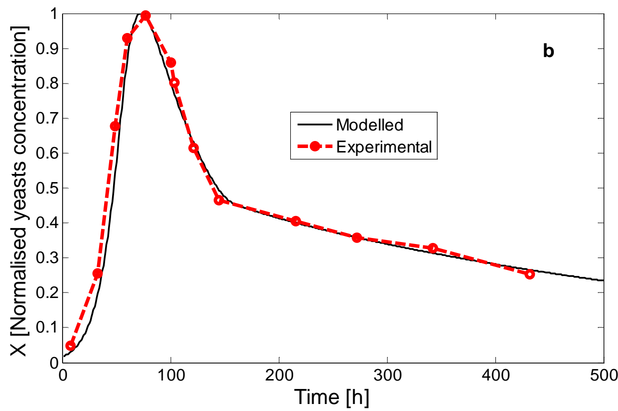


Figure 3: Normalised Viable Cells Profiles: Modelled and Experimental Fermentations: (**a**) FER1 (Max.= 92.3·10^6 cfu mL^{-1}) and (**b**) FER2 (Max.= 109.74·10^6 cfu mL^{-1}) both of them at 296±1K

Figure (4a), presents the model predictions and experimental results for fermentations performed at a predefined temperature profile, Fig. (4b), fixed from biochemical considerations on yeasts growth and yeast-related aroma compounds.

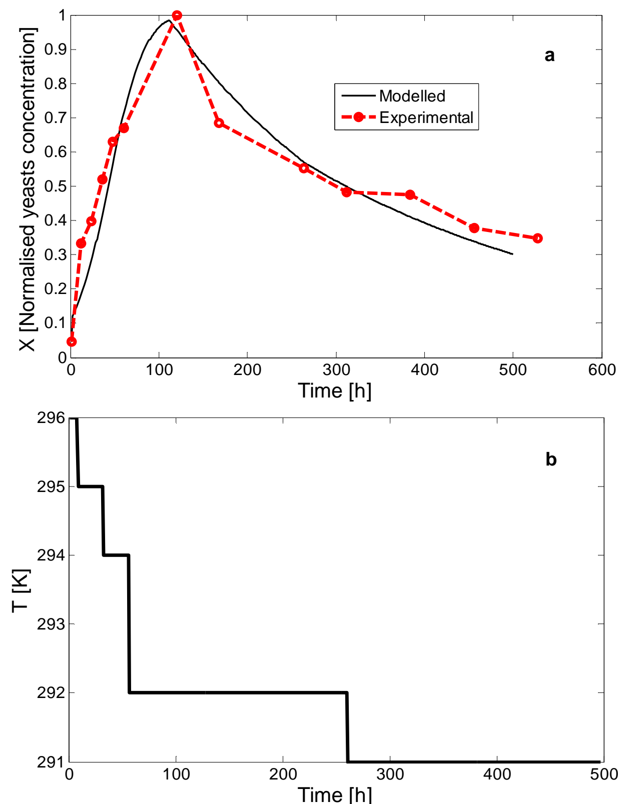


Figure 4: (**a**) Normalised Viable Cells Profiles: Modelled and Experimental Fermentation FER3 (Max.= 63.5·10^6 cfu mL^{-1}), at (**b**) a Specific fermentation temperature profile (296-291K)

Furthermore, results in Fig. 4, constitute the effective model validation since that it allows to predict the viable yeasts population when an optimal temperature profile is stated.

Table 1, illustrates a quantitative comparison of the obtained results in Figs. 3 and 4. According to Scaglia, Aballay, Mengual, Vallejo, and Ortiz (2009): firstly, it is used the mean absolute error (*MAE*, Eq. 12) that also has been used to predict biomass in this case,

$$MAE = \frac{\sum_{1}^{n} \left| X_{\text{mod}} - X_{\text{exp}} \right|}{n} \tag{12}$$

n is the number of experimental data, X_{mod} the predicted value of biomass (cells concentration) and, X_{exp} the experimental one.

Subsequently, the effectiveness of the presented model was assessed by means of the percentage mean error (*ME%*, Eq. 13) with respect to the experimental range of the variable expressed by its maximum value ($X_{\text{exp,max}}$); this, also regards the fermentation progress and its control (Malherbe, Fromion, Hilgert and Sablayrolles 2004).

$$ME\% = \frac{MAE}{X_{\text{exp, max}}} \cdot 100 \tag{13}$$

Lastly, in Table 1, it is exposed that both errors are into a typical maximum limit in biotechnology and process engineering of 10% with respect to data range of variable biomass, which is compensated with an experimental measurement error of about the similar value. Fundamentally, the predicted profiles do not show appreciable time retards with respect to the experimental data and achieves an enhanced precision by estimating biomass compared to own (Ortiz, Aballay, and Vallejo 2006), and other first-principles models like the ones of Coleman, Fish and Block (2007), and Phisalaphong, Srirattana, and Tanthapanichakoon (2006), respectively. This fact was attained with an additional critical variable as the temperature and the new parameters in the proposed model. Hence, it would be possible to apply it in control algorithms to track with proximity desired fermentation trajectories without significant delays in the control actions. Such characteristic is particularly essential during winemaking process, since a delayed control action on variables, such as temperature or pH, can generate a sluggish or stuck fermentation or the degradation in organoleptic properties of wine.

In addition, the model can be used at industrial scale with some adaptation, given that, other non-isothermal models developed from lab-scale alcoholic fermentations have been validated or tested with good performance, or highlighted their possible adaptation, taking into account scale-up effects (Phisalaphong Srirattana, and Tanthapanichakoon 2006; Colombié, Malherbe, and Sablayrolles 2007, Malherbe, Fromion, Hilgert and Sablayrolles 2004; Coleman, Fish, and Block 2007). In the work of Zenteno, Pérez-Correa, Gelmi, and Agosin (2010), the model was validated for a 10 m^3 industrial tank.

Table 1: Comparison between Simulated and Experimental Results for Viable Cells Concentration

Fermentation	MAE [10^6 cfu mL^{-1}]	ME%
FER1	0.4405	4.7722
FER2	0.232	2.1262
FER3	4.1527	6.5397

5. CONCLUSIONS

A first-principles model, for non-isothermal alcoholic fermentations in wider conditions of winemaking temperature, has been presented in this work. Since the bioprocess is strongly affected by temperature in aroma and flavour production, the final wine quality depends on monitoring and controlling on this variable. Therefore, the model obtained consist of mass balances, predicting state variables (viable cells, substrate and ethanol concentrations, and CO_2 released), coupled with an energy balance of the system. The latter is done by means of cellular growth and death parameters, and the $CO_{2\ (95)}$ parameter, all of them in function of temperature in an interval from 10 to 40°C.

The developed model has been satisfactorily validated via simulation with published and own experimental data, showing a proper behaviour to predict cellular growth kinetics at constant and variable predefined temperature profiles. This allows disposing of a reliable model to: approximate state variables trajectories and propose advanced control and optimization strategies.

The model validation reaches to lab-scale winemaking fermentations. It is possible to use it at industrial scale, in that case, it may be necessary include some aspects not considered such as: mixing of the fermentation mass and spatial concentration gradients, heat transfer, etc.

In addition, other topics will be included in next contributions, such as: to track other variables of the bioprocess as, substrate and ethanol concentrations, CO_2 released, density and/or pH; to show an extensive sensitivity study for model variables and parameters; to improve parameter estimation with artificial intelligence tools; to make efforts to reduce the winery cooling requirements even though the process demands specific cooling protocols to maintain low temperatures that protect the wine quality.

ACKNOWLEDGMENTS

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APPENDIX

Table 1: Coefficients and parameters values from the isothermal fermentation model of Scaglia, Aballay, Mengual, Vallejo, and Ortiz (2009), used in the present non-isothermal model for three fermentations.

Description		Unit	Value*		
Fitting Coefficient					
			FERT	FER1	FER3
a	-	-	1.276	1.319	1.276
b	-	-	1.242	1.282	1.242
c	-	-	1.276	1.276	1.276
d	-	-	1.152	1.152	1.152
e	-	-	1.355	1.355	1.355
A	-	-	5.622	3.119	5.622
B	-	-	93.02	93.02	93.02
C	Volume of fermenting mass per substrate mass	m^3 kg^{-1}	0.001	0.001	0.001
E	Volume of fermenting mass per formed cells and time	m^3 kg^{-1} hr^{-1}	1.01 $\cdot10^{-4}$	1.65 $\cdot10^{-4}$	1.01 $\cdot10^{-4}$
F	Specific rate of substrate consumpti-on for cellular maintenan-ce	kg kg^{-1} hr^{-1}	0.01	0.01	0.01
G	CO_2 released per formed cells	kg kg^{-1}	13.2	8.073	13.2
H	CO_2 released multiplied by time per formed cells	kg hr kg^{-1}	1440	880.7	1440
I	Similar to G	kg kg^{-1}	1.8	1.8	1.8
Kinetic and Yield Parameters					
Ks	Saturation coefficient in Monod's equation	kg m^{-3}	2.15		

β	Coefficient in Verlhurst's equation	m^3 kg^{-1} h^{-1}	$4.5 \cdot 10^{-3}$	$3.65 \cdot 10^{-3}$	$4.5 \cdot 10^{-3}$
$Y_{X/S}$	Formed cells per consumed substrate	kg kg^{-1}	0.04	0.061	0.04
$Y_{CO2/P}$	Carbon dioxide yield coefficient based on ethanol	kg kg^{-1}	0.775		

*Values for fermentation FER2 are not shown here.

Table 2: Initial conditions, coefficients and parameters used in the proposed non-isothermal model for three fermentations.

Description		Unit	Value*		
Initial Conditions					
			FERT	FER1	FER3
X(0)	Viable cells concentrat-ion (yeasts)	M-cfu m^{-3}**	$2 \cdot 10^6$		
S(0)	Substrate concentrat-ion	kg m^{-3}	200	208.5	226.2
$CO_2(0)$	Carbon dioxide evolution	kg m^{-3}	0		
P(0)	Ethanol concentrat-ion	kg m^{-3}	0		
T(0)	Bioreactor temperature	K	***	296	296
$t(0)$	Time	h	0		
$\mu_m(0)$	Maximum specific cellular growth rate	h^{-1}	$6.67 \cdot 10^{-2}$	$1.09 \cdot 10^{-1}$	$1.09 \cdot 10^{-1}$
$K_d(0)$	Specific cellular death rate per Kelvin degree	h^{-1}	$1.8 \cdot 10^{-3}$	$8.1 \cdot 10^{-3}$	$8.1 \cdot 10^{-3}$
$CO_{2,(95)}(0)$	CO_2 released between 85-95% of the maximum CO_2 released	kg m^{-3}	(*)	(*)	(*)
L(0)	-	-	1		
M(0)	-	-	1		
$Q(0)$	Exchanged heat between the fermenting mass and the cooling jacket	W	1.25	1.18	(**)
Maximum Value Achieved					
X_{max}	Viable cells concentrat-ion (yeasts)	M-cfu m^{-3}	$195 \cdot 10^6$	$92.3 \cdot 10^6$	$63.5 \cdot 10^6$
Fitting Coefficients					
f	-	-	$4.23 \cdot 10^{-7}$		
g	-	-	$6.41 \cdot 10^{-4}$		
h	-	-	0.3873		
i	-	-	116.9		
j	-	-	17631		
k	-	-	$1.06 \cdot 10^6$		
l	-	-	$2.7 \cdot 10^{-7}$		
m	-	-	$4.1 \cdot 10^{-4}$		
n	-	-	0.2479		
o	-	-	74.89		
p	-	-	11299		
q	-	-	$6.81 \cdot 10^5$		
Physical-chemical and Kinetic Parameters					
ρ_r	Density of the fermenting mass	kg m^{-3}	998.204		
C_{pr}	Specific heat of the fermenting mass	W·h kg^{-1} K^{-1}	1.01684		
V_r	Volume of the fermenting mass	m^3	0.003		
$Y_{H/CO2}$	Energy due to the carbon dioxide released by the bio-reaction	W·h produc-ed/kg of CO_2 releas-ed	310.3748		
γ	Maximum cellular growth rate per Kelvin degree	$h^{-1}K^{-1}$	1.08	1.66	0.83
ΔG_d	Gibbs free energy change of the fermentatio n reaction	kJ $kmol^{-1}$	1916.9		
E_a	Activation energy for cell growth	kJ $kmol^{-1}$	1928.37		

E_d	Activation energy for cell death	kJ $kmol^{-1}$	$1.7 \cdot 10^5$	$1.7 \cdot 10^5$	$1.789 \cdot 10^5$
$K_{d,0}$	Specific cellular death rate per Kelvin degree	h^{-1}	$3.1 \cdot 10^{26}$	$1.2 \cdot 10^{27}$	$1.2 \cdot 10^{27}$
$CO^*_{2(95)}$	CO_2 released between 85-95% of the maximum CO_2 released at constant temperature	kg m^{-3}	94	97.5	97.5
R	General gases constant	kJ $kmol^{-1}$ K^{-1}	8.309		

*Values for fermentation FER2 are not shown here.
**Millions of Colony Forming Units per cubic meter.
***Different constant temperatures: 288, 293, 298, 303 and 308K.
(*) Idem to $CO^*_{2(95)}$ values.
(**) Values according to temperature trajectory required.

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