Executive Summary of the Thesis

Analysis and experimental validation of mathematical models of wine fermentation

Laurea Magistrale in Automation and Control Engineering - Ingegneria dell'Automazione

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1. Introduction

In the last decade, the Italian wine industry experienced a relevant international expansion. The wine industry is in second place for energy consumption in the food industry [2] despite the sustainability issue has become more and more important for the Italian and foreign market. It is therefore of paramount importance to implement sound oenology methodologies, i.e., *precision* oenology approaches, which aim to spur the vinification process re-engineering possibly in a data-driven perspective.

The thesis falls into the framework of the technological cluster *ALL4INNOVATION*; the purpose is to assist Italian wine companies in this *technological transformation*, with the scope of improving wine quality and minimizing energy consumption.

The thesis aspires to lay the foundations for the implementation of a predictive model for the alcoholic fermentation of the *Amarone della Valpolicella DOCG* wine. The work consists of data analysis and modelling of the fermentation process. The pursued objectives are (i) prediction of fermentation kinetics and (ii) their optimization through the manipulation of temperature as a control variable.

To build a structured database, a sampling plan and an experimental apparatus are devised. A physical-based mathematical fermentation model describing the dynamics of sugar, ethanol, nitrogen, yeast, and oxygen is studied and its parameters are identified based on the conducted experiments. The model will be finally used to propose a strategy to minimize the energy consumption.

2. Winemaking Overview

Alcoholic fermentation is the anaerobic transformation of sugars, mainly glucose and fructose, into ethanol and carbon dioxide, and is carried out by the yeast [5]. The latter needs significant amounts of YAN (yeast assimilable nitrogen) in order to increase its population during fermentation process and to complete the fermentation. As a matter of fact, with low values of YAN the probability of troubling fermentations increases. Another important factor that affects the process is the temperature. It acts basically on the yeast activity: the higher the temperature, the more the yeast activity increases the fermentation rate. Red wines are typically fermented between 25℃ and 30℃. The troubling fermentations are called sluggish or stuck. The

reasons why problems during the process arise can be various [5]: too high sugar concentrations, extreme temperature, complete anaerobiosis, nutrient deficiencies, etc. This thesis case study is based on the *Amarone della Valpolicella DOCG* wine, from *Sartori* winery. It is a dry, full-bodied and complex wine characterized by a particular phase in its process: the dehydration. At the end of this phase, the sugar concentration reach values around 280–300 $\mathrm{g\,L^{-1}}$ and an alcohol potential close to 17%.

3. Instrumentation, Data Collection and Analysis

3.1. Sampling Plan

We have identified two types of samples: grape must and industrial must. These samples are composed of *Amarone* grapes respecting prescribed grape variety proportions. The experimentation is developed in five distinct periods ((1-4) belonging to the various stages of the drying phase, and (5) to the industrial phase), and ten grape suppliers were involved. The project is developed studying the fermentation at different scales, spanning from 350 mL (nanovinification), to 9 L (microvinification) up to the industrial one, at the *Sartori* winery, of 150 hL. The available analyses were:
- Ethanol profiles, measured during the whole fermentation process;
- Initial conditions for sugar and YAN;
- Final conditions of produced ethanol and sugar.

3.2. Must Preparation

The grapes are manually destemmed and then pressed with a hydraulic press. Then, 80 $\mathrm{mg\,L^{-1}}$ of Potassium Metabisulfite is added to avoid wine oxidation. The mixture is then kept in a cold room at 3℃ for about a week in order to simulate the winery maceration. After these days, the maceration mixture is filtered to separate the liquid from the solid part. The mass is heated until a temperature of 15℃ and 20 $\mathrm{g\,hL^{-1}}$ of an amino-acid-nitrogen-based complex activator (nutrition) are added. At this moment, 20 $\mathrm{g\,hL^{-1}}$ of hydrated yeast are inoculated.

3.3. Nanovinification

The nanovinification experiments allow for the evaluation of the fermentation kinetics and in particular the evolution of the alcohol content. This system uses bottles with a capacity of 0,5 L equipped with sensors which detect the pressure inside the bottle as a consequence of the production of CO_2. An equation correlates the pressure with the produced alcohol content. This experiments are carried out both with grape samples and with industrial must.

3.4. Microvinification

This experiment is performed in 13 L jars equipped with a heating/cooling system with the possibility to withdraw samples during the fermentation. This vinification has been only executed with the industrial must samples.

3.5. Experimental Results

The fermentations related to the grape samples nanovinifications are characterized by extreme variability. We have observed that the higher S_0 the higher is the alcohol content of the corresponding wine, and the higher the YAN the higher is the fermentation rate.
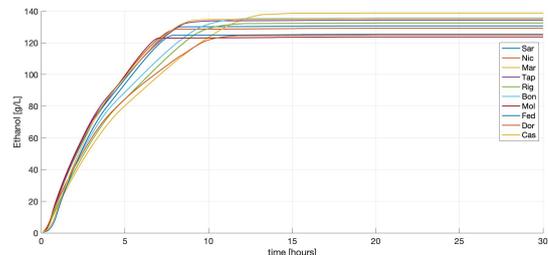
Figure 1: Kinetics of the fourth sampling period experiments.

Considering experiments having similar S_0 values, we have appreciated considerably high fermentation rate and lower fermentation times when the ratio $R = \mathrm{YAN}_0/S_0$ assume larger values. On the contrary, with low R values, the fermentation process is usually slower.

The fermentations related to the industrial must samples, for both nanovinifications and microvinification, resulted stuck and sluggish (the sugar final concentration is above 30 $\mathrm{g\,L^{-1}}$). This is totally reasonable given the high initial sugar concentration and the low initial nitrogen concentration. Moreover, we could observe the
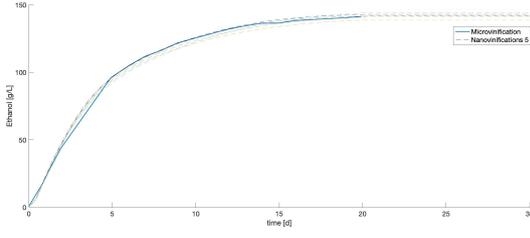
Figure 2: Kinetics of the fifth sampling period experiments, comparing nanovinifications and microvinification.

same behavior in the two experimental procedure kinetics.

4.   Fermentation Model

The adopted mathematical model has been formulated by Schenk & Schulz [4]. The state variables X, E, S, N, and O_2 represent yeast, ethanol, sugar, yeast assimilable nitrogen and oxygen concentrations, respectively.

$$\dot{X} = \mu(N,T)\frac{S}{K_{S1}+S}\left(\frac{O_2}{K_O+O_2}+\epsilon\right)X \quad (1)$$
$$- k_d X - \phi(E)\,X$$

$$\dot{E} = \beta_{max}\frac{S}{K_{S2}+S}\frac{K_E(T)}{K_E(T)+E}\,X \quad (2)$$

$$\dot{S} = -k_2\dot{E} - k_3\,\mu(N,T) \quad (3)$$
$$\frac{S}{K_{S1}+S}\left(\frac{O_2}{K_O+O_2}+\epsilon\right)X$$

$$\dot{N} = -k_1\mu(N,T)\frac{S}{K_{S1}+S} \quad (4)$$
$$\left(\frac{O_2}{K_O+O_2}+\epsilon\right)X$$

$$\dot{O}_2 = -k_4\mu(N,T)\frac{S}{K_{S1}+S}\frac{O_2}{K_O+O_2}X \quad (5)$$

The functions $\mu(N,T)$, $\beta(N,T)$ and $K_E(T)$ represent the fermentation dependence on temperature variations.

$$\mu(N,T) = \mu_{max}(T)\,\frac{N}{K_N+N} \quad (6)$$

$$\beta(S,T) = \beta_{max}(T)\,\frac{S}{K_S+S} \quad (7)$$

$$K_E(T) = -K_{E1}T + K_{E2} \quad (8)$$

The fermentation kinetics are described by Michaëlis-Menten equations. The specific grow rates μ_{max} and β_{max} are linearly dependent on the temperature:

$$\mu_{max}(T) = \mu_1 T + \mu_2 \quad (9)$$

$$\beta_{max}(T) = \beta_1 T + \beta_2 \quad (10)$$

The main peculiarity of this model is its precise modelling of the death of yeast cells, i.e.,

$$\phi(E) = (0.5+\frac{1}{\pi}arctan(k_{d1}(E-tol))) \quad (11)$$
$$k_{d2}(E-tol)^2$$

Here, tol is the tolerated ethanol concentration, k_{d1} and k_{d2} are parameters associated to the death of yeast cells due to ethanol exceeding the tolerance tol. This term makes sure that the evolution of the yeast population has a death phase after the stationary one. Other phenomena, that can cause the death of yeast cells, are described by $k_d \cdot E$ in equation (1).

The term ϵ allows to represent the fact that nutrients are consumed by the yeast even when there is no oxygen [4].

K_N and K_O are the half-saturation constants associated to nitrogen and oxygen, respectively. K_{S1} and K_{S2} are two saturation constants associated to the part of sugar used for yeast activity and to the other needed for the production of ethanol, respectively. At the same time, other two yield coefficient k_2 and k_3 associated to these two effects of the sugar are needed.

5.   Parameter Identification and Validation

Two parameter identification phases have been carried out in this thesis. One considered nanovinification experiments carried out using grape samples; the other, considered the nanovinification experiments performed in the last sampling period. The latter results have been used in the optimization study presented in Section 6.

3

5.1. Estimation 1

The strategy for the model identification consists of estimating the parameters over the ten experiments of the fourth drying period (see Section 3). We decided to perform this identification since the initial conditions of these experiments were similar to the real winemaking conditions, in terms of initial sugar and yeast assimilable nitrogen. The parameter estimates are presented in Table 1, along with their initial values.

Parameters	Initial Val.	Estimate
μ_1	0.08	2.1524
β_1	0.3371	0.8687
K_{S1}	33.35	72.145
k_2	1.2	1.8327
k_3	15	11.389
K_{S2}	4.3262	3.6417
K_N	0.1156	0.8270

Table 1: Parameters estimation with their initial values.

The identification can accurately predict the fermentation performance of both ethanol and sugar kinetics for all the ten considered experiments. We observed small accuracy losses with low values of YAN_0/S_0. Given the good performances, we used the other periods experiments as validation. Eight among the other thirty experiments were considered outliers because of their troubling fermentations. A good portion of experiments were predicted with good accuracy:

- Considering all the experiments, the portion of "good prediction" is 72%;
- Considering the experiments in which $R \in$ [0.5 - 0.8] (real winemaking conditions [3]) the percentage increases to 90%;
- The model found difficult to predict experiments with very low values of R. In fact, for R < 0.5, 41.2% of the experiments are "well predicted".

The result of this identification operation are quite satisfactory; in fact, the model can predict fermentation kinetics with good accuracy in a wide range of initial condition. Accuracy losses in fermentation predictions may depend on a vast multitude of variables and behaviors.

Therefore, the reasons why certain experiments have not been "well predicted" cannot be inferred only by the initial value of sugar and YAN. Further study considering other variables (i.e., amino acids, vitamins, acids) will improve the accuracy of the model.
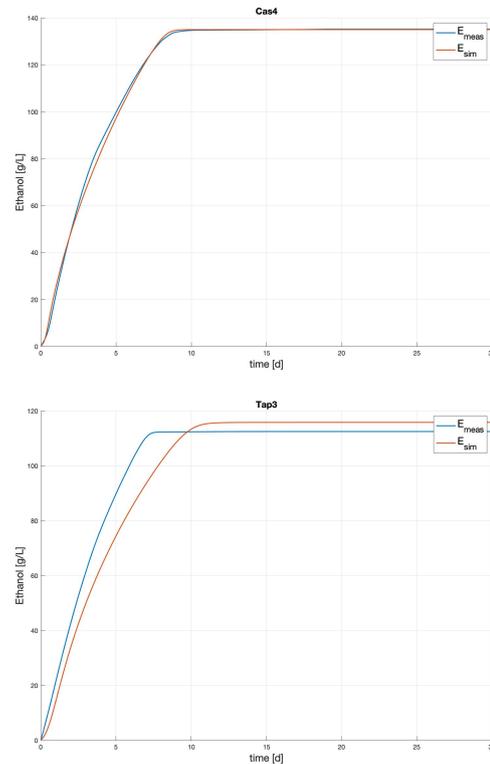
Figure 3: Example of "good" and "bad" predictions.

5.2. Estimation 2

A dedicated identification procedure for the experiments conducted with industrial must samples was performed because of its extreme initial conditions. This strategy consists of identifying a new set of parameters over the mean curve of the five nanovinification experiments carried out using the industrial must (sampling period 5). The initial values of the parameters were the ones obtained in the previous estimation phase, except for k_d; its initial value was taken from [4]. The new estimates are listed in Table 2. Considering this identification phase, the model can predict the experiments kinetics (nanovinification 5 and microvinification) with satisfactory results. This identification will be used in the following optimization study.

Parameters	Initial Val.	Estimate
μ_1	2.1524	0.5011
β_1	0.8687	1.3170
K_{S1}	72.1450	33.4210
k_2	1.8327	0.8798
k_3	11.3890	87.1650
K_{S2}	3.6417	3.8915
K_N	0.8270	0.0905
k_d	0.0100	0.1268

Table 2: Parameters estimation with their initial values.

6. Optimization Strategy

The purpose of this work was to compute a temperature profile aimed to optimize the process in order to achieve, at the same time, lower tracking error, lower fermentation time, and lower energy consumption. This procedure aims to calculate the optimal temperature profile (denoted $\vec{T_u}$) by optimizing an open-loop objective function over the entire time horizon. The considered cost function is the following:

$$ J = \gamma_1 \sum_t \Delta E^2(t) + \alpha t_f + \gamma_2 \sum_i \Delta T^2(i) \quad (12) $$

where $\Delta E(t)$ and $\Delta T(i)$ are:

$$ \Delta E(t) = (E_{ref}(t, t_f) - E_{sim}(t, \vec{T_u})) \quad (13) $$

$$ \Delta T(i) = (T_{ext} - T_u(i)) \quad (14) $$

In the cost function J, $E_{sim}(t, \vec{T_u})$ is the simulated ethanol, $E_{ref}(t, t_f)$ is the desired ethanol, t_f is the instant at which the fermentation ends, $T_u(i)$ is the temperature inside the jar (control input, i.e., $\vec{T_u} = \{T_{u_1}, ..., T_{u_N}\}$), T_{ext} is the external temperature (chosen to be equal to 22 °C). Furthermore, γ_1, γ_2 and α are suitable weights. Constraints have been added regarding the maximum and minimum temperature values (15 °C and 28 °C), and its variation ($\Delta T_{max} = \pm 2$ °C). The ideal ethanol curve E_{ref} was constructed through a fitting procedure in order to be function of the ideal fermentation time, i.e., it is itself a function of the free variable t_f:

$$ E_{ref}(t, t_f) = \frac{(\frac{20}{t_f}t)^b}{(\frac{20}{t_f}t)^b + c^b} a \quad (15) $$

where $a = 149.9$, $b = 1.644$ and $c = 3.646$.
A weight was assigned to each of the three terms that form the cost function (12), and the best trade-off was found via a trial-and-error procedure. The chosen weights are: $\gamma_1 = 10$, $\gamma_2 = 5$ and $\alpha = 10^6$.
The input variable profile $\vec{T_u}$ is a piece-wise constant curve. Therefore, the temperature may assume a different value at each sampling step of length τ_k. The latter has been chosen to be variable; more specifically, it is shorter at the beginning of fermentation, when the kinetics are faster, and longer towards the end, when the fermentation rate is almost zero. The result of the optimization procedure is depicted in Figure 5.
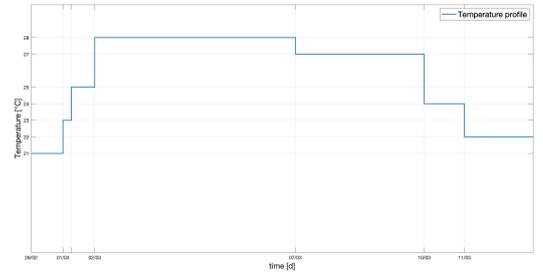
Figure 4: Optimal temperature profile.

It is likely that the low initial YAN content and the contribution given by the high temperature (28 °C against 24 °C of previous fermentation), have inhibited the fermentation on the fourteenth day. In fact, according to [1], when YAN_0 is low, the yeast grows rapidly consuming almost all the nitrogen; high temperatures tend to intensify this behaviour.
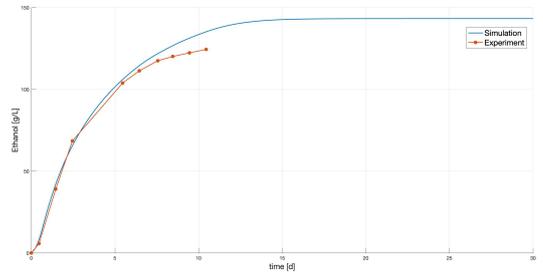
Figure 5: Comparison between simulated kinetic and experimental result.

The goal of the optimization study was to investigate the behaviour of fermentative kinetics and to validate our identification of the mathematical model under conditions of variable tem-

perature on a small-scale tank. Given the low excitation of the data from the previous experiments with respect to temperature, we cannot be certain that the estimated parameters work under different operating conditions. However, the outcome of this study can be a basis for improving the identification of the model parameters in the future.

7. Conclusions

The work consisted of data analysis and modelling of the fermentation process of *Amarone della Valpolicella DOCG* wine. The objectives pursued were (i) prediction of fermentation kinetics, and (ii) their optimization through the manipulation of the control variable, i.e., the temperature.

To build a structured database, a sampling plan and an experiments apparatus were devised. A physical-based mathematical fermentation model describing the dynamics of sugar, ethanol, nitrogen, yeast, and oxygen was studied and its parameters were identified based on the experiments conducted.

The model studied in this thesis cannot be considered the proper one to predict the fermentation performances for an *Amarone* wine. However, this thesis has laid the methodological grounds for its development in the medium term. The critical point in this experimentation was to produce and collect large quantities of data in order to train mathematical models.

Another great limitation has been the insufficient excitation of the fermentation process with respect to the temperature profile. Conducting experiments in non-isothermal conditions and at different scales will allow data to be more informative and avoid practical identifiabilty issues. This will also lead to improvement in the mathematical models which will be used for automatic fermentation control purposes.

In the framework of the *Precision Winemaking Model* project, it will be necessary to overcome the technological and organizational limitations. Considering our experience, some improvements are here suggested:

- Sampling plan improvements;
- YAN analysis methods accuracy;
- Instrumentation upgrading;
- Mathematical modelling improvements;
- Techological support introduction.

8. Bibliography

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