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# Analysis and experimental validation of mathematical models of wine fermentation

Master Thesis in
Automation and Control Engineering


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# Abstract

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 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 of *Amarone della Valpolicella DOCG* wine. The objectives pursued 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 experiments apparatus are devised. A physical-based mathematical fermentation model describing the dynamics of sugar, ethanol, nitrogen, yeast, and oxygen is studied and its parameters will be identified based on the conducted experiments. The model will be finally used to propose a strategy to minimize the energy consumption.

**Keywords:** data analysis, predictive models, model identification and validation, optimization, amarone DOCG wine, wine alcoholic fermentation.

# Abstract in lingua italiana

Nel corso dell'ultimo decennio, il settore vitivinicolo italiano ha conosciuto una rilevante fase di espansione, soprattutto sul fronte della crescita internazionale. L'industria del vino è al secondo posto per consumi energetici nel settore alimentare nonostante siano numerosi i Paesi dove l'attenzione alla sostenibilità assume sempre maggiore importanza. È quindi fondamentale implementare una nuova metodologia detta *enologia di precisione* per l'avvio in sicurezza del re-engineering, in una logica data-driven, del sistema di gestione del processo di vinificazione.

Questa tesi è inserita nel contesto del cluster tecnologico *ALL4INNOVATION*; lo scopo è quello di aiutare le imprese vitivinicole italiane nella *trasformazione tecnologica*, migliorando così la qualità del vino e minimizzando i consumi energetici.

La tesi intende porre le basi per l'implementazione di un modello predittivo della fermentazione alcolica del vino *Amarone della Valpolicella DOCG*. Il lavoro consiste in uno studio di analisi di dati e di modellizzazione del processo di fermentazione del vino *Amarone della Valpolicella DOCG*. Gli obiettivi perseguiti saranno (i) predizione della cinetica fermentativa e (ii) ottimizzazione della stessa attraverso la manipolazione della variabile di controllo temperatura.

Per creare un database strutturato, sono stati ideati e applicati un piano di campionamento e un sistema di esperimenti di fermentazione di laboratorio. È stato studiato un modello fisico-matematico per la fermentazione che descrive le dinamiche di zucchero, etanolo, azoto, lievito e ossigeno e i suoi parametri sono stati identificati basandosi sugli esperimenti condotti. Infine, su questo modello sarà basato uno studio di ottimizzazione per minimizzare i consumi energetici.

**Parole chiave:** analisi di dati, modelli predittivi, ottimizzazione, identificazione e validazione, amarone DOCG, fermentazione alcolica del vino.

# Contents

# 1 | Introduction

## 1.1. Context and Motivation

The winemaking processes are characterized by a huge amount of variables that may affect the grapes. In particular, climatic conditions, altitude, type of terrain, and many other conditions may result in very different grapes' conditions. This high variability of the raw material imposes appropriate modifications also of the vinification process. In fact, depending on the initial conditions of the grapes, the winemaking process may require different yeast types, nutrition addition and may lead to different fermentation times. The wine production chain is unique, because of the necessity to manage variations of different types of products in terms of characteristics, identity, geographical typicality, and variability of individual vintage.

The wine industry still widely adopts traditional methodologies, despite present-day technology allows the management of the vinification process and to reducing variability of the wine characteristics.

Current projects, e.g., the so-called *Precision Winemaking Model* one, are devised for the increasing market demands in terms of authenticity, sustainability, salubrity, and quality of the wine industries. These demands cannot be satisfied solely thanks to the oenologist's talent and experience anymore. On the contrary, nowadays the improvement in the process management and the introduction of more sound methods (defined as *precision*) are necessary for this industry. Therefore, a *technological transformation* of the vinification process is necessary for the wine industry; new technologies for data collection and management, data support systems, data banks, and predictive models are requested.

This thesis lays the foundations for the implementation of a predictive model for the alcoholic fermentation of the *Amarone della Valpolicella DOCG* wine. The project lies in the framework of the technological cluster *ALL4INNOVATION*, in favour of a group of Italian wine companies. The project has been developed to "implement a new *precision* winemaking methodology". Specifically, the goal is to help these companies with

the introduction of more sound practices, defined *precision*, that lead to data-validated decisions and to significant energy and cost savings.

The *grape fermentation wine* chain must be considered in a unitary fashion in order to collect the necessary data to train mathematical models. An experiment-based system, consisting on nanovinifcations, microvinifications and industrial vinifications, must therefore be developed to investigate different operational conditions (e.g., yeast strains, nutritional strategies, fermentation temperatures, etc.), different agronomic techniques (which may lead to different chemical and organoleptic parameters of wines), and energy saving.

This experimental system has the objective to create the database for developing predictive models of: sustainability (energy saving), authenticity (isotopic, metabolic, gene), quality (shelf life and sensory), food safety (organic and inorganic contaminants), and corporate liability (compliance with standards and laws).

The expected benefits of these methodologies are:

1. encouraging innovation and development of new products
2. reducing operating costs through the optimization of resources (raw materials, energy, water, waste, etc.)
3. predict fermentation performances in order to plan the production process in relation to market demands.
4. predict fermentation problems that may affect wine quality.

## 1.2. Contribution of the Thesis

This thesis proposes a study concerning data analysis and model identification on the *Amarone del valpolicella DOCG* wine, produced by the *Sartori* winery (VR).

The objectives of this thesis are:

1. Predict fermentation kinetics starting from the initial conditions of the must after the pressing phase, with particular interest in fermentation time.
2. Study the optimization of the fermentation kinetics on a laboratory-scale vinification by acting on the temperature profile applied to the fermenting must.

For achieving the former objective, a detailed study of the fermentative kinetics of wine is necessary. In this perspective, it is necessary to have a sufficiently large amount of

data on fermentation kinetics to cover the number of variables that concur to the alcoholic fermentation process. To analyze the biological behavior of this wine, the *Ever - Italiana Biotecnologie* company (VI) has made available its equipment for winemaking experiments. Various chemical analyses, similar to those of the winery, were carried out also by the *Vassanelli Laboratory* (VR).

To build a structured database, a sampling plan and an experiment apparatus were implemented. We actively worked on their conception and development: starting from the sampling phase in the *Sartori Fruttaio*, leading up to the pressing and the managing of the fermentation tank at *Ever - Italiana Biotecnologie*. This had the objective to create a sound database based on which it was possible to carry out the study of the mathematical model.

Since the thesis project is part of a broader study, many choices were made for purposes external to that of the thesis. In particular, the winemaking tests were done using two yeasts (*Inverno 1936* and *Vulcano*) made available by *Ever*. However for the implementation, identification, and optimization of the mathematical model, only one of the two yeasts was considered (*Inverno 1936*).

As far as the second objective is concerned, a sound mathematical predictive model will be needed for devising strategies aiming to reduce the energy consumption. Specifically, the energy consumption in cooling/heating tanks during the fermentation process is investigated. The purpose is to lay the foundations for possible future studies on the economically optimal control of fermentation kinetics.

In general, this thesis creates a methodological basis to implement other mathematical-predictive models suitable to help wine companies to apply the PDCA cycle (Plan-Do-Check-Act) to the winemaking process. Particular interest is given to the planning phase of production processes (vineyard and winery) to obtain optimal results and prevent risks in authenticity, sustainability, quality, food safety and legality areas.

### 1.2.1. Partners and Strategic Choices

This project was possible thanks to a convergence of interests between many stakeholders listed below:

**Sartori Winery.** The study tackles the flagship product of *Sartori* winery, i.e., the *Amarone della Valpolicella DOCG* wine. Thanks to *precision* winemaking approach, they want to stand out based on objective data as a distinctive element with respect to competitors;

**Winegrowers.** This project is viewed, by winegrowers, as an opportunity to obtain and enhance certifications in the sustainability field (with reference to standards like *3R*, *Equalitas*, *SQNP*, etc.);

**Research and Supply Chain Partners.** This study is an opportunity for these partners to study and apply innovative solutions already tested on other simpler processes to one of the most complex production processes, winemaking. The latter are *Ever - Italiana Biotecnologie* (VI), *Vassanelli Laboratory* (VR), *APRA* (AN), and various Research Partners (e.g., *Università di Verona - Viticoltura ed Enologia - Dipartimento di Biotecnologie* (VR)).

## 1.3. Thesis Structure

The thesis is structured as follows:

- Chapter 2 presents a winemaking overview to introduce the reader to the wine essential concepts. The basics are briefly discussed for a better understanding of the following chapters, together with the main variables affecting the alcoholic fermentation kinetics.
- Experimental choices, vinification experiments and analysis that allowed the data collection are presented in Chapter 3. The types of samples that have been collected are two:
  - grape samples: taken during the dehydration phase by the *Sartori* drying rooms, also known as *Fruttai*;
  - must samples: collected after the pressing phase of the *Sartori* winery.

  The experiments that are carried out with these samples are of two different types and are called:
  - *Nanovinification*: tests in 0.5 L bottle;
  - *Microvinification*: tests in 13 L jar.

  The limitations of vinification on laboratory operating conditions will be presented as a result of these types of experiments' analysis. In fact, the procedures that have been carried out in laboratory try to imitate the winery conditions and to simulate the industrial vinification in order to study the fermentation kinetics. It is clear that it is not possible to replicate exactly the conditions of industrial winemaking; however, the laboratory allows to make numerous tests and to analyze a

number of parameters that would hardly be analyzed in winery. Together with these winemaking tests, a variable's analysis system was set up. In fact, *Ever - Italiana Biotecnologie* has made available its tools for measuring the concentrations of ethanol, sugars, vitamins, acids and amino acids on musts and wines. The analyses carried out can be divided into two groups:

  - Static analyses: they were manually carried out by the staff of *Ever - Italiana Biotecnologie* on micro-samples of must and wine, collected before and after the winemaking experiments.
  - Dynamic analyses: the latter are the analyses performed during the fermentation tests; in these, only ethanol was measured.

  This system of "sampling-vinification-analysis" allowed to build a database of fermentation kinetics on which it was possible to identify a suitable mathematical model.

- After a detailed review of the state-of-the-art we present, in Chapter 4, a critical review and comparison of selected fermentation physical-based mathematical models. The aim is to select one of these models and then proceed to parameters identification over the experimental data.
- The large number of tests (160) allows a good identification of the model and contains great variability of initial conditions. The identification procedure is presented in Chapter 5.
- The optimization study, presented in Chapter 6, concerns an optimization problem with the aim of calculating the optimal temperature profile to minimize:
  - Consumed energy;
  - Fermentation time;
  - Tracking error from a desired ethanol profile.

  The optimal profile is applied to the heating/cooling system of a jar and the ethanol kinetics were observed.

# 2 | Winemaking Overview

This chapter is dedicated to illustrate in details the process of alcoholic fermentation in winemaking, with particular focus on the *Amarone della Valpolicella DOCG* wine. The main steps of winemaking are illustrated in Figure 2.1, starting from the grape vintage and ending with the wine bottling.

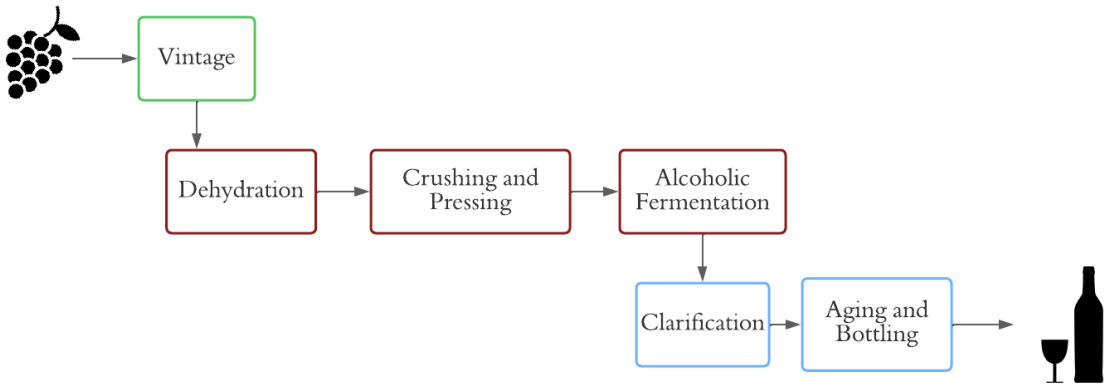


Figure 2.1: Main winemaking process steps.

## 2.1. Alcoholic Fermentation

Alcoholic fermentation is the anaerobic transformation of sugars, mainly glucose and fructose, into ethanol and carbon dioxide [24]. This process, which is carried out by yeast and some bacteria, can be summarised by following reaction:

$$\underset{\text{Hexoses}}{C_6H_{12}O_6} \longrightarrow \underset{\text{Ethanol}}{2CH_3CH_2OH} + \underset{\text{Carbon Dioxide}}{2CO_2}$$

As this reaction proceeds, a number of other biological and chemical processes take place. Indeed, several other compounds are produced, listed in Section 2.3.

At the beginning of the winemaking process, the grape juice may contain several species of yeast, depending on multiple factors such as grape variety, ripening stage, treatments, climatic conditions, viticultural practices, and development of mold or fungal plagues.

*Saccharomyces Cerevisiae* is the predominant kind of yeast because of its greater resistance to high ethanol concentration. Some other yeasts may also be present in the grape juice and even in the wine itself, which may cause some organoleptic defects and influences the final composition of wine in a positive or negative way. By inoculating selected strains of dry yeasts (*Saccharomyces Cerevisiae*) the winemakers try to prevent developing of undesirable yeasts.

### 2.1.1. Yeast Development

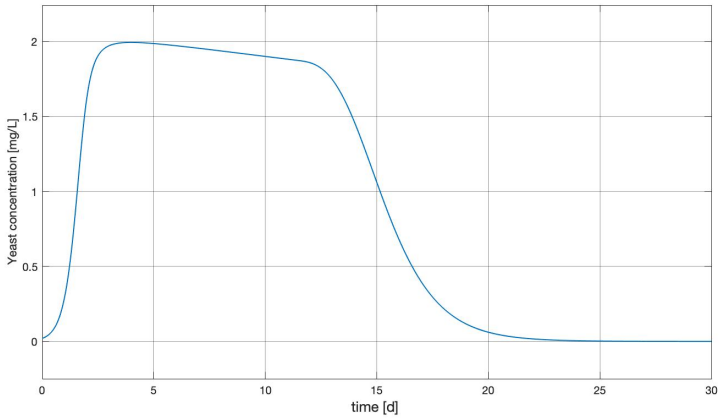


Figure 2.2: Example of simulation of yeast kinetic during alcoholic fermentation.

At the beginning of the fermentation, the yeasts start to metabolize the sugars and other nutrients present in the must to obtain energy and increase their population [24]. In the first hours, during the so called *Latency phase*, the number of cells does not increase, it is necessary for the cells to adapt to the new environmental conditions. Once the yeast cells adapt, they start to grow: in this *Exponential Growth phase*, the population begins to increase and in this phase temperature plays a key role together with concentration of ammonia, amino acids and other nutrients; this phase lasts from 3 to 6 days. After the growth stops, because of the deficiency of some nutrients, now the *Quasi-stationary phase* begins: the number of yeast cells remains almost constant. After 2 - 10 days, the *Decline phase* begins and the yeast cells start dying, because of the lack of nutrients and also because ethanol and other substances, produced during alcoholic fermentation, are toxic for them; this phase lasts until the cells have almost completely disappeared. The objective is to avoid stuck and sluggish fermentation (discussed in Section 2.2), which can be done maintaining the population of viable yeast at sufficient levels until all the fermentable sugars have been fully consumed. It is important to highlight that the concentration of cells (number of cells per milliliters of grape juice) remains the same for

the entire process: indeed the yeast cells dies, but they does not disappear in the must. This phenomenon will be considered in some fermentation models (presented in Chapter 4), which make this distinction between yeast and active yeast.

### 2.1.2. The Importance of Nitrogen

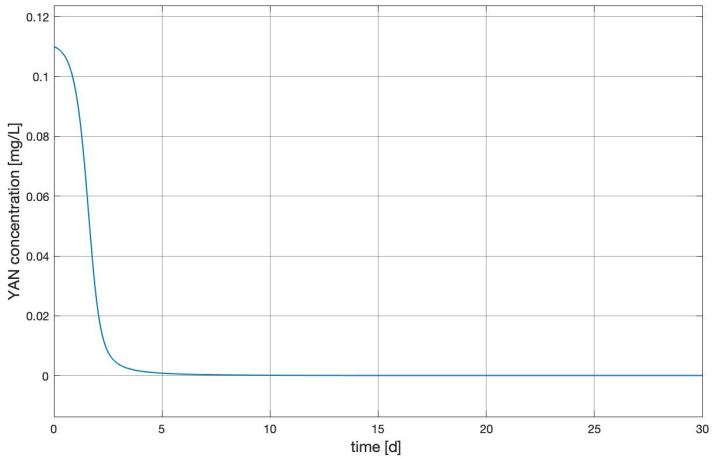


Figure 2.3: Example of simulation of nitrogen kinetic during alcoholic fermentation.

*Saccharomyces Cerevisiae* needs significant amounts of assimilable nitrogen to increase its population during fermentation process [24]. Inside the grape juice there is a variety of nitrogen compounds (such as ammonia, amino acids, peptides, proteins, etc.), but only some of them can be assimilated by the yeast. *Saccharomyces Cerevisiae* can only use ammonia and amino acids, with the exception of proline, as an assimilable source of nitrogen. These substances are called YAN, *Yeast Assimilable Nitrogen*, (APA, *Azoto Prontamente Assimilabile*, in Italian). Grape juice is relatively poor in ammonia and amino acids and, with low values of YAN the probability of stuck or sluggish fermentations increases. For this reason, winemakers use to supplement grape juice with a *Nutrition* composed basically on ammonium salts. The YAN requirement for a the alcoholic fermentation depends on the yeast strain and the potential alcoholic degree, that depends on the initial sugars in the grape juice. In contrast, too high concentration of nitrogen can lead to the production of toxic substances. For this reason, nitrogen must be supplemented carefully and taking into account the initial YAN concentration and the potential alcoholic degree of the wine.

### 2.1.3. The Temperature Effect

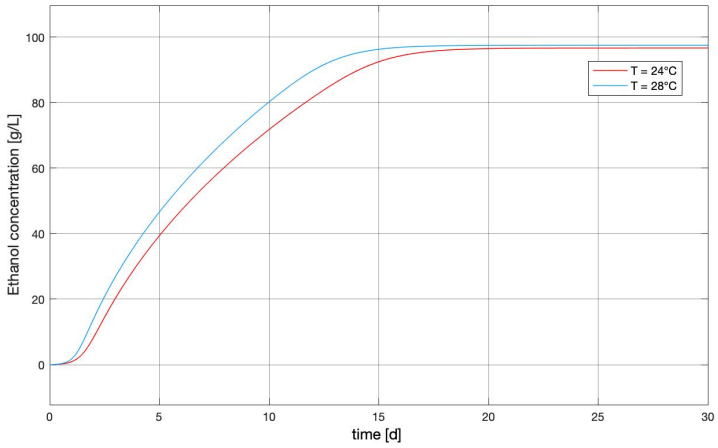


Figure 2.4: Two examples of simulation of ethanol kinetics with two different temperatures of 24 ℃ and 28 ℃.

Alcoholic fermentation is an exogenous chemical reaction, so when heat is applied, fermentation rate is higher [24]. With increasing temperature, the yeast will convert faster the grape's natural sugars to alcohol: in fact, between 15 °C and 25 °C the fermentation rate is doubled when the temperature is increased by approximately 8 °C. In Figure 2.4, the fermentation rate difference can be appreciated when fermentation occurs at two distinct temperatures (24 °C and 28 °C). When the temperature is higher, the fermentation ends 5 days before. Nevertheless, too high fermentation temperatures may reflect on the wine specifics. Indeed, high temperatures might kill yeast cells or prevent the population from growing. On the contrary, lower fermentations temperatures help to preserve the flavors and the desired characteristics of the wine. Red wines are typically fermented between 20 °C and 30 °C. This range results in richer colors, good levels of tannin and fruit flavors. Moreover, the temperature profile is also important. In fact, an increase of several degrees during the fermentation process greatly changes the fermentation kinetics.

## 2.2. Stuck and Sluggish Fermentations: Causes and Solutions

At the end of the process, alcoholic fermentation might become too slow. It can happen that yeasts reduce the sugar consumption and before all the fermentable sugars have been completely metabolised, fermentation stops. When this happens, two problems may arise:

- The wine can be not finished and actions must be taken to finish it.
- There is high risk of bacterial spoilage.

Various studies [24] analyzed the reasons of stuck and sluggish fermentations. Here the possible causes and solutions of these issues are summarized:

1. High sugar concentration: excessive levels of sugar in the must may inhibit yeasts in some cases. Moreover, a high concentration of ethanol can be a serious problem for the complete consumption of sugars, especially during the final stages of fermentation. In this case, using yeast with high ethanol resistance is recommended.
2. Extreme temperatures: when the process starts at too low temperature the population of yeast cells may have problems to grow. Also, if the temperature is too high (more than 30℃) the risk of stalled fermentation is very high. Moreover, rapid changes of temperature may provoke serious problems in fermentation. For these reasons, nowadays fermentation temperature needs a thermic control.
3. Complete anaerobiosis: oxygen is necessary, without it yeast will not grow and may adapt itself to the environmental conditions. For this reason, aeration is recommended, especially during the exponential growth phase.
4. Nutrient deficiencies: the lack of some nutrients in the must can bring to serious issues during the process, because nitrogen, vitamins, minerals, etc. may be deficient in grape juice. For this reason, yeast activators are usually added in wineries: the nutrient consists of ammonium salts (phosphate and/or sulphate), thiamine and their application is certainly very useful. Moreover, adding nitrogen is more effective if it is done more than once and if it is combined with aeration.
5. Presence of anti-fungal substances: sometimes grape juice can contain residues substances that may affect alcoholic fermentation. To avoid this, inspections at vineyard level are indispensable.
6. Antagonism between microorganisms: the different microorganisms which are present in grape juice compete for nutrients. Indeed, sometimes autochthonous yeast or even bacteria can grow and they can be the causes of deviations and even stuck and sluggish fermentations.

All these causes can prevent alcoholic fermentation from developing correctly. However, it is rare to find a single responsible for stuck and sluggish fermentation: usually the cause is a synergistic combination of some of them. Nevertheless, in case of problems in a fermentation tank, winemakers must act as soon as possible: with aeration and

inoculating yeast the issue may be solved. In fact, if fermentation stops, the yeast must be reinoculated. The succes of the inoculum is based on the choice of the yeast and the way it is preadapted to ethanol.

## 2.3. Other Subproducts of Alcoholic Fermentation

Alcoholic fermentation is not only the process that transform sugars into ethanol, but it is also a complex process that leads to an equally complex product, wine. Indeed, sugars are transformed mainly into ethanol but also into other subproducts, which can contribute negatively or positively to wine quality. Moreover, alcoholic fermentation also implies the transformation of other compounds present in the grape juice which have a high influence on wine quality.

- Diacetyl, acetoin and 2,3-butanediol: they are not present in significative amounts, indeed, they do not have a real effect on the aroma. When lactic acid bacteria are present, they can considerably increase their concentrations, affecting the organoleptic characteristics of the wine.
- Ethanal: this compound is reduced for the most part into ethanol, but some little quantities may be released into the wine. It gives off the aroma of oxidized wine, although in alcoholic fermentation, this compound is produced in a small amount. High concentration of ethanal can be found in some wines, obtained by aging the wine under a film of *Saccharomyces Cerevisiae*, that produces ethanal from ethanol.
- Acetic acid: this compound is the main volatile acid of wine. High concentrations of it give off a vinegar odour and a disagreeable sensation in the mouth. Indeed, *volatile acidity* is one of the most important analytical parameters in oenology. This compound can be produced by yeast, but normally *Saccharomyces Cerevisiae* only produce small quantities of acetic acid without problems during alcoholic fermentation. However, in case of stuck and sluggish fermentations the production of this acid can increase.
- Higher alcohols: normally their presence is below the limit of detection, but they are the precursors of some esters, which have a large sensory impact.
- Esters: the first group (acetates of higher alcohols) gives off different odours, such as rose (*phenylethanol acetate*), banana (isoamyl acetate) or glue (ethyl acetate). The second group (*esters of fatty acids and ethanol*) is responsible of a fruity aroma.
- Succinic acid: it has significant effect on wine acidity. The yeasts also release into

the wine several other acids such as lactic acid, fatty acids, isobutyric acids, etc. in low concentrations.

## 2.4. Amarone della Valipolicella DOCG Wine

The *Amarone* wine is produced in Valpolicella, a region situated along the province of Verona's foothills. The official birth of *Amarone* wine dates back to 1936, in the wine cellars of Villa Mosconi in Novare di Arbizzano, Negrar [1]. The Denominazione di Origine (DOC) "Valpolicella" was created in 1968, both for *Valpolicella Recioto* (a sweet wine) and *Valpolicella Recioto Amarone* (a dry wine), because *Amarone* was considered to be the "child" of *Recioto*. The peculiarity of this kind of wine are the varieties used in the grape juice, and the dehydration technique that is unusual for the classic winemaking process.

### 2.4.1. The Dehydration Techniques

Dehydration phase is a key factor in the *Amarone* production chain. This procedure allows to develop the typical organoleptic characteristics of this wine [1]. The spaces dedicated to this phase are big warehouses called *Fruttai*, see Figure 2.6. Here, the grapes are stored in pallets so that water inside them can evaporate; dehydration ends when the grapes have lost the 30-40% of their weight. This process lasts about 2 months. The aim of this procedure is to increase the sugar and so the potential ethanol, influencing the alcoholic fermentation and leading to a dry, full-bodied and complex wine. The controlled dehydration systems used today are essentially made up of dehumidifiers and vents (Figure 2.7) working at ambient temperatures with important air changes. The humidity levels are kept between 60% and 70%. This variable, together with temperature, plays a key role in this procedure.

### 2.4.2. Vinification

At the end of the dehydration process, the sugar concentration has reached values around $280 - 300\ \mathrm{g\,L^{-1}}$ and an alcohol potential is close to 17%. The grapes are then crushed and destemmed, eliminating clusters attacked by vulgar Botrytis (fungi).

After adding nutrient to the must (the importance of this inoculation is explained in Section 2.2), fermentation can start: at this moment, the external temperature is very low, the grapes may enter the tanks when temperature is around $0\,^{\circ}\mathrm{C}$. Now the choice of yeast is extremely important, since the conditions are not favourable with this low temperatures and high sugar levels. A good yeast for *Amarone* wine needs to be able

to start fermentation at low temperatures, a high alcohol production (∼18 °C) and a low degradation of malic acid (which is normally present in small quantities).

Figure (2.6) Grape stored in pallets in *Fruttaio*.

Figure (2.7) Vents to keep the desired temperature.

With this particular low-temperature vinification, there are two types of maceration: the first, before the fermentation, is a cold maceration of crushed grapes and the second is the maceration that takes place during the fermentation process. The process usually starts 8˘10 *days* after crushing and may last around 30 *days*. Then a second step consists of the tumultuous fermentation, that goes from a 3–4% to a 13–14% alcohol content, and it lasts 10˘15 *days*. Finally, the final phase takes place, the alcohol content goes from 14% to 17%. The latter is the most important phase, where heat can be applied and that lasts $5-7$ *days*. The fermentation temperature should be between 22 °C and 26 °C. In this project, fermentation temperature is kept at 24 °C, as it can be seen in Chapter 3. Finally, residual sugar (3-4 $\mathrm{g\,L^{-1}}$) can be found in the wine and the fermentation is generally completed in barrels.

### 2.4.3. Grape Varieties

Figure 2.7: Example of Corvinone variety grape, stored in *Fruttaio*.

The key factor for the grape variety choice is their ability to shrivel [1]. This characteristic mainly depends on genetics and production techniques. The variety that may be found in the grape blend for *Amarone* wine are listed below:

- *Corvina*: the most important variety of the grape blend for the production of Valpolicella wines. In fact, the new DOCG (*Denominazione di Origine Controllata e Garantita*) rules accept a *Corvina* percentage between 40% and 80% (including the *Corvinone* variety). It is a vigorous late-flowering variety, fairly cold hardy.
- *Rondinella*: this variety is cold hardy and drought-resistant. It is characterized by a low sensitivity to fungal diseases. For this reason it is suitable for the dehydration process. *Rondinella* can be used to between 5% and 30%, according to the new DOCG rules.
- *Corvinone*: similar to *Corvina*, it is a different variety, the new DOC rules stated that can form up to 50% of the grape blend. It is an ideal variety for dehydration for its thick skin and juicy berries.
- Optional varieties: other red varieties, among those that are authorized and rec-

ommended for the province of Verona, may be added to the blend with an overall maximum of 15%, and 10% per single variety. For example *Molinara*, *Croatina*, *Merlot*, *Cabernet* etc.

### 2.4.4. Sartori Amarone Winery

The process of industrial vinification begins with the unloading of the boxes of dried grapes inside the collection tank, and then in the crusher-destemmer. On the newly obtained grape must, the following must be added:

- $10\,\mathrm{g\,hL^{-1}}$ of potassium metabisulphite previously dissolved in water;
- $20\,\mathrm{g\,hL^{-1}}$ tannin having antioxidant action, previously soluble 1:10 in warm water.

The fermentation temperature, regulated by two external jackets containing refrigerant glycol, is set to 4 °C, so as to leave the grapes crushed and destemmed in maceration for about a month, avoiding the onset of undesired spontaneous fermentation. This period of maceration allows further extraction from the skins of tannins and polyphenolic compounds to further increase the dry extract of the finished wine. As already mentioned in Section 3.7, in this phase the mass stratifies and remains uneven: this behavior makes the vinification at industrial scale very different from those dealt with in Chapter 3. It is important to note that any analysis of the total sugar content, and therefore potential ethanol, can be considered reliable from 4-5% alcol content, since at this stage the carbon dioxide produced allows the mixing of the total mass.

At the end of the maceration phase, the glycol temperature is set to 20 °C to heat the pressed and avoid thermal shock to the yeast, which after a few days is rehydrated according to internal protocol. To the total mass one adds:

- $20\,\mathrm{g\,hL^{-1}}$ of yeast specific to *Amarone*, tolerant to high concentrations of ethanol and able to express the maximum sensory potential of grapes;
- $4\,\mathrm{g\,hL^{-1}}$ of specific activator for rehydration and start fermentation;
- $20\,\mathrm{g\,hL^{-1}}$ of an amino-acid-nitrogen-based complex activator (nutrition) to feed the yeasts and avoid situations of reducing stress.

To the rehydrating yeast a quantity of must is gradually added such as to bring the *pied de cuve* (fermentation starter) to a quantity of about 10% of the total mass. In this way the yeast settles in its final growth environment and does not suffer stress when it is inoculated in the fermenter. Now, the fermentation phase begins. At this point two daily air-exposed pump-overs take place. At the first pump-over, $10\,\mathrm{g\,hL^{-1}}$ of tannin is

added. The glycol temperature is set to 15 °C to obtain a fermentation temperature of approximately 25 °C. Then:

- At about 6% alcohol, $20\,\mathrm{g\,hL^{-1}}$ of ammonium-based nitrogen activator is added;
- At about 12% alcohol, $20\,\mathrm{g\,hL^{-1}}$ of specific activator is added. This helps the yeast to complete the fermentation in the final stage, more critical both from the point of view of ethanol, and from the point of view of the presence of toxic substances produced by the yeast in firming.

Finally, when the total quantity of sugar is reduced to (3-4 $\mathrm{g\,L^{-1}}$), the wine is separated from the pomace and it is decanted into steel tanks where the long refinement phase begins.

# 3 | Instrumentation, Data Collection and Analysis

## 3.1. Introduction to the Experimental Setup and to Data Collection

As discussed in Chapter 2, a massive number of elements play a role in the alcoholic fermentation process. In fact, as more formally described in Chapter 4, the dynamics of the fermentation phase can depend on multiple factors that influence the transformation of sugar in ethanol. For example, a faster production of alcohol in the grape must may depend on the type of used yeast, but also on the quantity of yeast assimilable nitrogen (YAN).

To study a similar process we need a large amount of experiments, trials and data. For these reasons, a structured method for data collection, based on a massive numbers of solid experiments, is needed.

A suitable experimental campaign has therefore been designed to examine a huge variability of operational conditions. Ten grape suppliers of *Sartori* winery were considered in order to explore as many different grape condition as possible. In fact, in the different vineyards, fruit conditions might be considerably different depending on various factors, such as terrain type, slope or sun exposure.

We decided to withdraw grapes in four different periods during the dehydration phase, starting from the last days of September until the middle of December. The sample times were equally distributed in this period, spaced nearly twenty days each. Moreover, we identified a fifth period for the withdraw of the industrial must samples.

Furthermore, we will study the fermentation at different scales, spanning from 350 mL (nanovinification), to 9 L (microvinification) to the industrial one, at the *Sartori* winery, of 150 hL. The data provided by the latter will not be used for this thesis purposes (for the reasons explained in Section 3.7), but will be useful for future works. Table 3.1 collects

the names of the different types of fermentation experiments that we carried out.

| Name | Quantity | Location |
|---|---|---|
| Nanovinification | 350 mL | *Ever - Italiana Biotecnologie* |
| Microvinification | 9 L | *Ever - Italiana Biotecnologie* |
| Ind.Vinification | 150 hL | *Sartori* winery |

Table 3.1: Processes of vinification and relative quantities and locations.

## 3.2. Instrumentation

### 3.2.1. Nanovinification Equipment

The nanovinification experiment allows for the evaluation of the fermentation kinetics and, in particular, of the evolution of the alcohol content. They were carried out using the ANKOM$^{RF}$ *Gas Production System*. This system uses of bottles with a capacity of 500 mL closed with caps (RF1 modules) equipped with sensors that, at regular intervals (every 30 minutes), detect the pressure inside the bottle, as a consequence of the production of $CO_2$. The ANKOM$^{RF}$ system records the pressure of the produced gas at regular basis until the end of fermentation. The increase in pressure is used to indirectly determine the alcohol content in the must. The relative production of alcohol content is calculated using the equation of the line $y = 5.2173x - 0.1442$ where $x$ is the cumulative pressure value. This equation correlates pressure and produced ethanol (with a correlation coefficient $R^2 = 0.9828$) and has been derived from the analysis of data obtained from a pool of more than three hundred experiments carried out by the *Ever - Italiana Biotecnologie* company, see Figure 3.1.

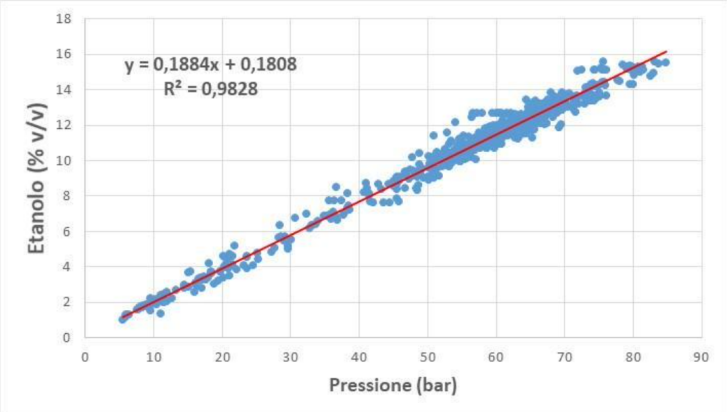


Figure 3.1: Correlation graph relating alcohol content and developed pressure.

The main advantage of the nanovinification experiments is availability of the measuring system. Since the pressure measurement is automatically collected every thirty minutes, the ethanol kinetic measurement is much more accurate with respect to the one obtained in the microvinification system (where the alcohol content is manually measured, and the number of data is considerably lower, see Section 3.5.2) or the methodology consisting of measuring the weight loss in the flask.

The bottles are placed in incubators equipped with agitators and with the possibility of setting the desired temperature. Considering the reduced bottle volume, the time that must needs to reach the desired temperature is of the order of a few minutes. In this case, using this particular equipment, it is not possible to withdraw samples during the fermentation. In fact, if the cap gets opened, the pressure measurement is compromised.


Figure 3.2: Bottles ready to start the fermentation with ANKOM$^{RF}$ caps.

### 3.2.2. Microvinification Equipment

The device shown in Figure 3.3 is called jar and is used for the microvinification process. It consists of a glass tank with capacity 13 liters where alcoholic fermentation takes place.

A heating/cooling system is also present. The latter is composed of an outer jacket surrounding the jar, in which water flows at approximately 10 °C. The presence of a resistor that heats the water before entering the jacket ensures that the must can also be heated up. The system has a temperature sensor and a monitor interface thanks to which a set-point can be imposed, so that the temperature can be kept constant at the desired value. An agitation system is also present, composed of a rotor that emulates the movements in industrial fermentation tanks. Importantly, it is possible to collect samples

of fermented must from which the percentage of produced alcohol and other features of interest can be measured (Figure 3.3, right panel). In this case, the measurements are done manually; therefore, the sample frequency can be chosen at will. In this work we have collected one sample per day. Only the alcohol content was measured in this work.

Figure 3.3: The jar for the microvinification, with the pipe and valve to withdraw samples.

### 3.2.3. Analysis Instruments

Besides the fermentation kinetics, other data concerning the initial and final composition of the must and wine were needed in order to set up the identification and optimization problems. In particular, we collected the measurements of initial sugar, yeast assimilable nitrogen, and final sugar concentrations. The samples of must, new wine in fermentation and finished wine, collected in the various stages of the experimental process, were subject to analysis from two different laboratories: *Vassanelli Lab* and *Ever - Italiana Biotecnologie*. Both laboratories are project partners: the former is a control laboratory responsible for commercial compliance and food safety analysis; the latter is a biotechnological research laboratory that hosted our experimentation. The analytical methodology used by the two laboratories to determine YAN values is different:

- *Ever - Italiana Biotecnologie* uses the HPLC (High-performance liquid chromatograph) technique for sugars, and an UPLC - MS/MS (Ultra-high performance liquid chromatography tandem mass Spectrometry) for the determination of the amino

acids from which the YAN value is directly derived.

- *Vassanelli Lab* uses the FOSS multiparameter analysis tool that provides both sugar and yeast assimilable nitrogen values. The analytical technique principle is the FTIR (Fourier Transform Infrared spectroscopy). The YAN content is measured by comparison with a database of enzymatic YAN values (as the sum of ammoniacal and amino nitrogen) and the value obtained on the sample.

The YAN values obtained by these methodologies are considerably different. For instance, in Table 3.2 the differences in the musts used for nanovinification 4 are shown.

| Supplier | UPLC/MS-MS | FOSS |
|---|---|---|
| Mol | 136 | 200 |
| Nic | 165 | 210 |
| Dor | 123 | 200 |
| Sar | 150 | 210 |
| Fed | 128 | 187 |
| Tap | 136 | 196 |
| Cas | 158 | 194 |
| Bon | 109 | 162 |
| Rig | 107 | 152 |
| Mar | 108 | 135 |

Table 3.2: Comparison between the YAN measuraments using UPLC - MS/MS and FOSS in the musts of the fourth period experiments. The unit of measure is $[\mathrm{mg\,L^{-1}}]$.

The UPLC technique allow to obtain more reliable data. Despite this, the YAN value obtained with FOSS was chosen for convenience. In fact, the costs, the times of analysis, and the availability of tools or internal competences are not compatible with the industrial practice. The two analytical techniques (FTIR and UPLC) also allow to measure the value of sugar concentration. Since they both are extremely reliable, we opted for those of *Vassanelli Lab*.

The FTIR technique also allow to measure the value of alcohol content. *Ever - Italiana Biotecnologie*, instead, employs an Alcolyzer Wine M (Anton Paar), which uses the NIR technique (Near Infrared spectroscopy). Because of the reliability of the methods, the comparability of data, the availability of both instruments in wineries, and the similarity of costs, we opted for the alcoholic content measurements obtained by *Ever - Italiana Biotecnologie* laboratory.

From these two laboratories, a number of further analytical parameters were obtained. However, the latter are not used in this thesis. Nevertheless, they give an important contribution to the project (see Chapter 1) and will certainly be used in future studies.

## 3.3. Sampling Plan

In this section the sampling plan is briefly described.
We have identified two types of samples: grape must and industrial must. The former is collected during the drying phase, while the latter immediately before the *Sartori* winery fermentation, see Section 2.4.4. The industrial must sample is the result of the grape pressing. The sampling plan follows the following guidelines:

- **Sampling periods** - To study the evolution of the grapes during the whole drying phase until the vinification, five instants were identified:
  - **Period 1**: beginning of the drying phase;
  - **Period 2**: $\frac{1}{3}$ of the drying phase;
  - **Period 3**: $\frac{2}{3}$ of the drying phase;
  - **Period 4**: end of the drying phase;
  - **Period 5**: beginning of the industrial fermentation phase.

  The time period between each sampling instant and the following one is about twenty days, as detailed in Table 3.3.
- **Variety of grapes** - As already mentioned in Chapter 2, *Amarone* wine is produced with a blend defined according to its production disciplinary. Therefore, each sample of grapes was taken respecting the prescribed proportions of grape variety: 60% Corvina, 20% Corvinone, 15% Rondinella and 5% minor varieties.
- **Suppliers selection** - Ten grape suppliers were selected, they are labelled as follows: “Bon”, “Cas”, “Dor”, “Fed”, “Mar”, “Mol”, “Nic”, “Rig”, “Sar”, “Tap”.
- **Sample quantity** - The amount of must needed for experiments and analyses is approximately 2 L. However, during the drying phase the grapes go through a process of dehydration and the weight decreases. In fact, to obtain 2 L of must, from 5 kg to 10 kg of grapes (depending on the dehydration level) are necessary.

Table 3.3 presents the samples collected and the weight loss data of the *Sartori* winery during the whole drying phase.

| Period | Date | Dehydration days | Sample | Sample size | Weight Loss |
|---|---|---|---|---|---|
| 1 | 24/09/2021 | 0 | Grape | 5 kg | 0% |
| 2 | 18/10/2021 | 24 | Grape | 7.5 kg | 15% |
| 3 | 15/11/2021 | 52 | Grape | 8.5 kg | 27% |
| 4 | 15/12/2021 | 82 | Grape | 10 kg | 33% |
| 5 | 13/01/2022 | - | Must | 50 L | - |

Table 3.3: Sampling phases for the experimental databank, weight loss measure is provided by *Sartori*.

## 3.4. Preparation of the Must

In this section the process that grapes and industrial must undergo before being vinified is described. We decided to adhere as much as possible to the vinification protocol of *Sartori* winery (see Section 2.4.4). These processes were conducted separately on each sample.

Concerning each grape sample, once collected and brought to the *Ever - Italiana Biotecnologie* laboratory, the grapes are manually destemmed (fruit and branches are separated) and pressed with an hydraulic press (see Figure 3.4), to obtain approximately 2 liters of must.

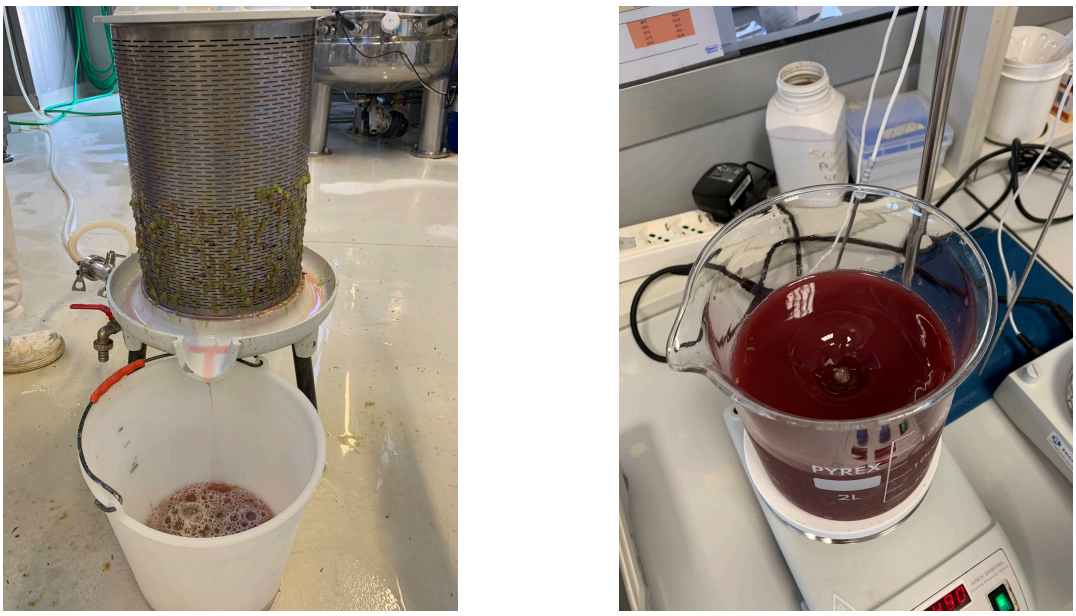


Figure 3.4: Hydraulic press used to obtain the must (left), must in agitation with nutrition added (right).

Then the laboratory maceration phase, which aims to emulate the one that is conducted in the winery, takes place: a quantity of the pomace equal to roughly half of the must volume is added to the liquid. 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 °C for about a week. In this phase, the red grape pomace adds flavors and color to the must (see Chapter 2). After these days, the maceration mixture is filtered to separate the liquid from the solid part. The latter is removed, and the must passes to the next stage of fermentation. The mass is heated until reaching a temperature of 15 °C and $20\,\mathrm{g\,hL^{-1}}$ of amino-acid-nitrogen-based complex activator (nutrition) are added. At this moment, $20\,\mathrm{g\,hL^{-1}}$ of hydrated yeast are inoculated, and the fermentation starts.

Concerning the industrial must sample, it consists of only the liquid part without pomace. The latter is first collected and brought to the *Ever - Italiana Biotecnologie* laboratory and then it is treated in the same way as the must after the laboratory maceration phase described above.

## 3.5. Experiments

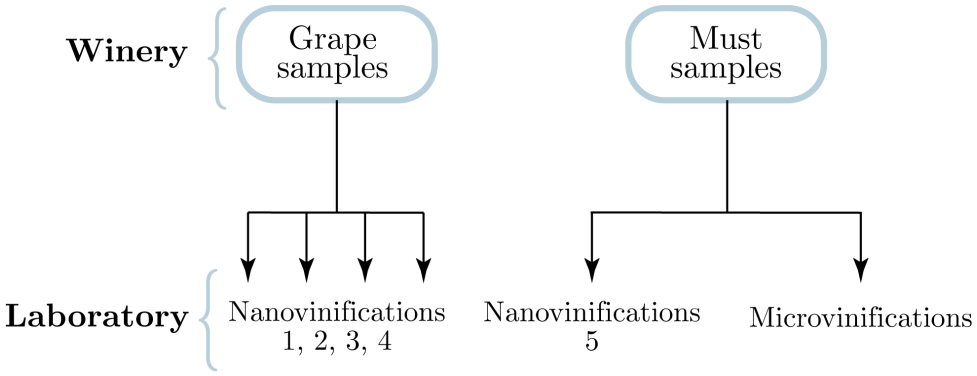


Figure 3.5: Scheme presenting the samples type, correlated with the performed experiments, the numbers after nanovinification correspond to the sampling periods.

### 3.5.1. Nanovinification


Figure 3.6: Bottles for nanovinification ready to start the fermentation.

As shown in Figure 3.5, the grape samples are used in nanovinification experiments. To avoid lack of data due to technical or other problems during fermentation, it was decided to run each experiment in duplicate. Therefore, for each sample, four nanovinification bottles were set up. Each of these bottles were filled with 350 ml of must: two of them were inoculated with the *Inverno 1936* yeast and two with the *Vulcano* one. Finally, the temperature of the incubators was set to 24 °C, as in the *Sartori* winery.

Since four periods of nanovinifications (corresponding to the nanovinifications 1,2,3,4 in Figure 3.5) are taken into account (i.e., four drying phases) and since we have used ten grape suppliers, the number samples is in total 160.
As far as the industrial must sample is concerned, it was decided to set up five nanovinifcations for each type of yeast, resulting in ten experiments (nanovinifications 5 in Figure 3.5). As already mentioned in Chapter 1, only the data coming from experiments with *Inverno 1936* yeast will be considered in this thesis.


Figure 3.7: Wine after nanovinification (left) and Wine samples (right).

### 3.5.2. Microvinification

Figure 3.8: Microvinification jar.

Only industrial must was used for these tests, as shown in Figure 3.5. Two types of experiments were conducted.
The first consists of a vinification at a constant temperature of 24 °C, as for nanovinification and, consequently, vinification in *Sartori* winery. The two types of yeast were used, one per jar.

The second experiment consists of a vinification at variable temperature. This vinification was used for the optimization study described in Section 6.2. The fermentation was run in duplicate: the same temperature profile and the same yeast (*Inverno 1936*) were used for both jars. For both experiments, each jar was filled with 9 litres of must.

## 3.6. Data Adjustment

The data concerning the evolution of the concentration of ethanol (i.e. the fermentation kinetics), produced by the nanovinifications and microvinifications have been provided. Then, data adjustment procedures have been performed in order to make them suitable for the identification and optimization software.

Regarding the nanovinifications in bottle, the operations are listed here.

- The ANKOM$^{RF}$ software detects the measurement every thirty minutes. To reduce the computational load, we decided to increase the sampling interval to two hours without loss of generality.
- We performed an ethanol measurement conversion from $E[\%]$ to $E[\mathrm{g\,L^{-1}}]$ to make it suitable for the considered model. As discussed, the conversion formula is: $E[\mathrm{g\,L^{-1}}] = E[\%] \cdot 10 \cdot \rho$, where $\rho = 0.789\,\mathrm{g\,cm^{-3}}$ is the ethanol density.
- Since the considered model (see Section 4.4.7) does not take into account the lag phase [19], it was decided to remove all ethanol data below $2\,\mathrm{g\,L^{-1}}$ [17].
- As already mentioned, every experiment has been done in duplicate. For each grape

supplier and for each period, instead of considering two separate fermentations, was decided to compute an average between the data collected during the two experiments. Since the ethanol mean error between the duplicates was on the order of maximum $1.68\,\mathrm{g\,L^{-1}}$, we assumed that there was no loss of generality in doing so. In Figures 3.9 and 3.10 the two experiments that have the maximum and minimum mean error, respectively, are shown. "Bon" and "Fed" are the name abbreviations of two of the the grape suppliers of *Sartori* winery.

- For the sake of identification and optimization, it was necessary to find the time instant in which the fermentation ends for each experiment. We have assumed that the fermentation can be considered terminated if the rate at which ethanol is produced is smaller than $0.05\,\mathrm{g\,L^{-1}\,h^{-1}}$.

For what concerns microvinifications in jars, no data adjustments were necessary.

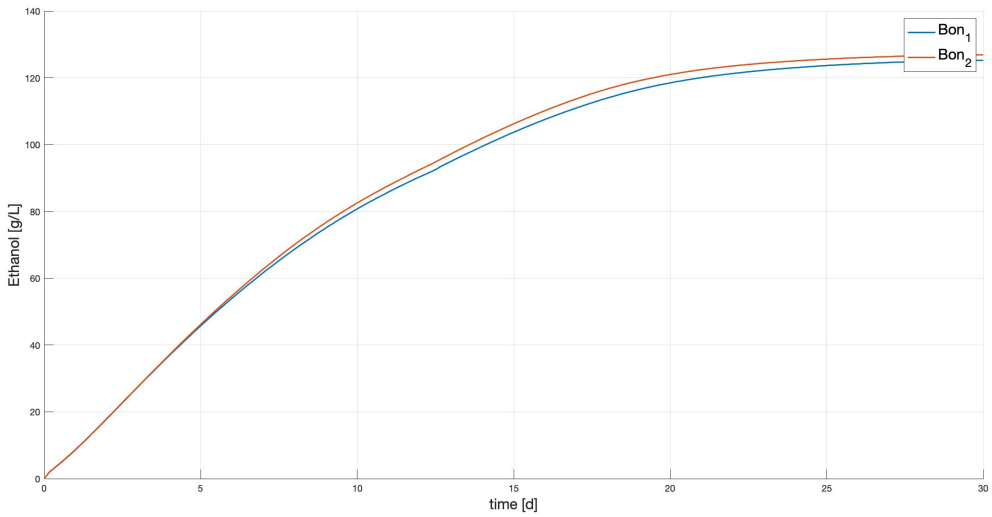


Figure 3.9: Experiments with the highest mean error equal to $1.68\,\mathrm{g\,L^{-1}}$.

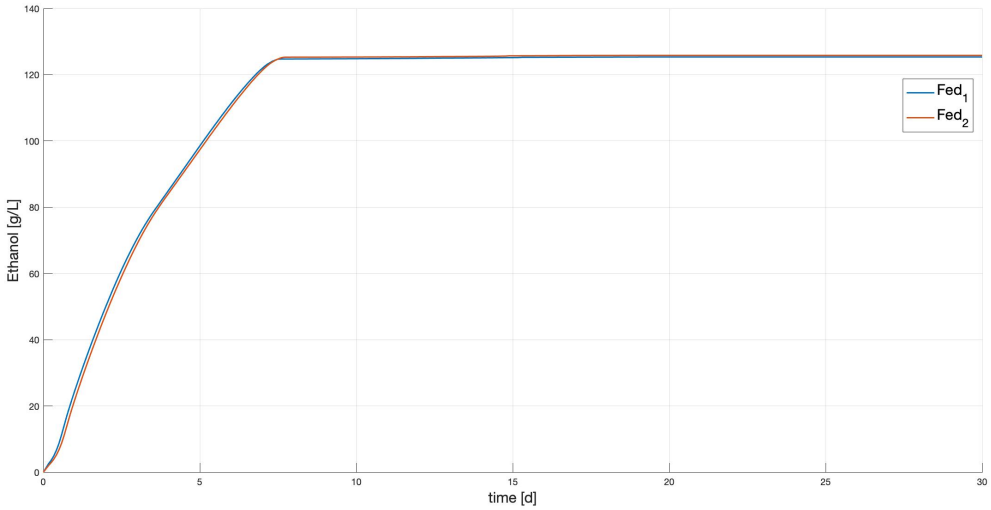


Figure 3.10: Experiments with the lowest mean error equal to $0.0036\,\mathrm{g\,L^{-1}}$.

## 3.7. Limitations of Small-Scale Vinifications

Most studies [17] on the kinetic parameters of wine fermentation have been performed at laboratory scale. However, the hydrodynamics of reactions in small fermenters change considerably with respect to those in large tanks (containing up to several hundred hectolitres of wine). In addition, considering non-isothermal fermentation, the laboratory facilities do not permit comparison with industrial vinifications.

In red winemaking, maceration represents the major problem and, on small scale, industrial conditions cannot be perfectly replicated. The hydrodynamic conditions in traditional red winemaking fermentation systems are highly specific and cannot be simulated in an adequate manner in laboratory fermentors. Therefore, the conditions should be studied with tank volumes of at least 100 L [16]. During vinification, the pomaces rise to the surface to form a cap, which becomes compact over time. This cap is only partly immmersed (approximately half). The upper part of the cap is highly heterogeneous in terms of both temperature (Schmid et al. [20]) and yeast concentration. Pumping over (or cap punching) is usually carried out to homogenize the substances in the tank. In studies of changes in $CO_2$ production rate in such fermentation conditions at 100 L scale, Aguera et al. [2], observed cap formation and the effect of cap punching on fermentation kinetics. They experienced a highly significant boost in $CO_2$ production rates after cap punching. This increase was nearly immediate, continuing over several hours, and it was higher when pumping over was carried out during the stationary phase. Cell population measurements indicated that this kinetics acceleration was mostly attributable to a trans-

fer of yeasts from the cap to the liquid phase, increasing the size of the cell population in the liquid (by more than 50%).

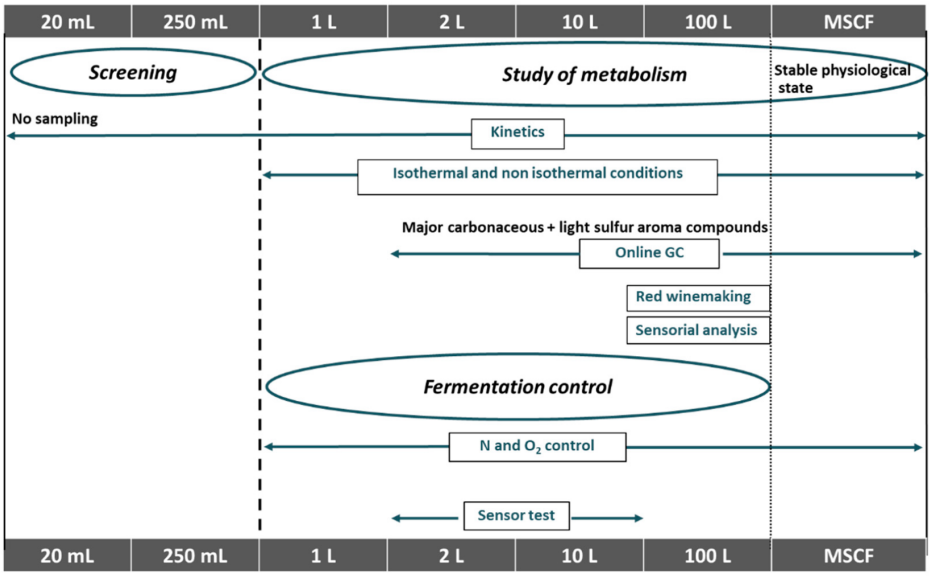


Figure 3.11: Choice of the fermentation scale and equipment required for answering different research questions, Figure from [16].

Mouret et al. [16] summarizes in the scheme shown in Figure 3.11 which experimental setup is needed to be organized to best answer different research questions. For what concerns the study carried out in our thesis, it can be noted that the fermentation kinetics can be an object of study at all scales. Fermentation control, instead, is only feasible with fermenters from 1 L to 100 L. Here, again, 100 L scale is especially appropriate for red wine fermentation [16]. Other studies objectives not treated here (for example study of yeast metabolism or sensorial analysis), can be observed.

What can be found in this scheme matches with the experiments that we have carried out. In fact, the nanovinification system in 0.350 L bottles was very effective and useful to study the kinetics of the various fermentations, as can be seen the following sections. Moreover, thanks to the 9 L microvinification system, in addition to the study of fermentative kinetics, it was possible to complete a optimization study on the of the vinification process, as can be observed in Chapter 6.2. However, for the reasons mentioned above, these fermentation systems are still small-scale and, therefore, it was not possible to compare them with the red wine fermentation process performed by the winery.

## 3.8. Experimental Results and Analysis

The results of the vinification experiments and the analyses on the collected samples are briefly collected in this section.
As discussed, for each available sample, we could measure the initial condition of sugar and the yeast assimilable nitrogen, the final ethanol concentration, and the fermentation time.

As explained in the previous sections, the experiments carried out are: nanovinifications 1-4 (with pressed grapes, divided in four periods), nanovinifications 5 (with industrial must), and microvinifications (with industrial must). Figures 3.12-3.15 and Tables 3.4-3.7 present the ethanol kinetics during the nanovinification phase together with the measurements of interests. Moreover, Figures 3.17, 3.18 and Table 3.8 show the same analysis and kinetics related to the industrial must.

### 3.8.1. Grape Sample Experiments

#### Nanovinifications 1

The must collected during the drying period 1 is characterized by low value of sugar and yeast assimilable nitrogen for all suppliers. For what concerns the kinetics in Figure 3.12, we can appreciate good fermentative performance for almost all the ten experiments, with a small loss in fermentation rate in only three of them. This issue might depend on the low ratio $R = \mathrm{YAN}_0/S_0$. Specifically, the ratio $R$ gives information about the initial quantity of YAN related to the initial sugar. The latter has shown, in this experiments, to affect significantly the fermentation kinetics: in fact, when $R$ takes values smaller than a certain value, we could observe lower fermentation rates (consequently, higher $t_f$). However, we cannot directly correlate the fermentation rate with this ratio. In fact, comparing the data labelled "Nic" and "Rig" (from Table 3.4), that display a similar value of $R$, we can notice that in the experiment with lower sugar, the fermentation rate is significantly higher ($0.772\,\mathrm{g\,L^{-1}\,h^{-1}}$ for "Rig" and $0.534\,\mathrm{g\,L^{-1}\,h^{-1}}$ for "Nic").

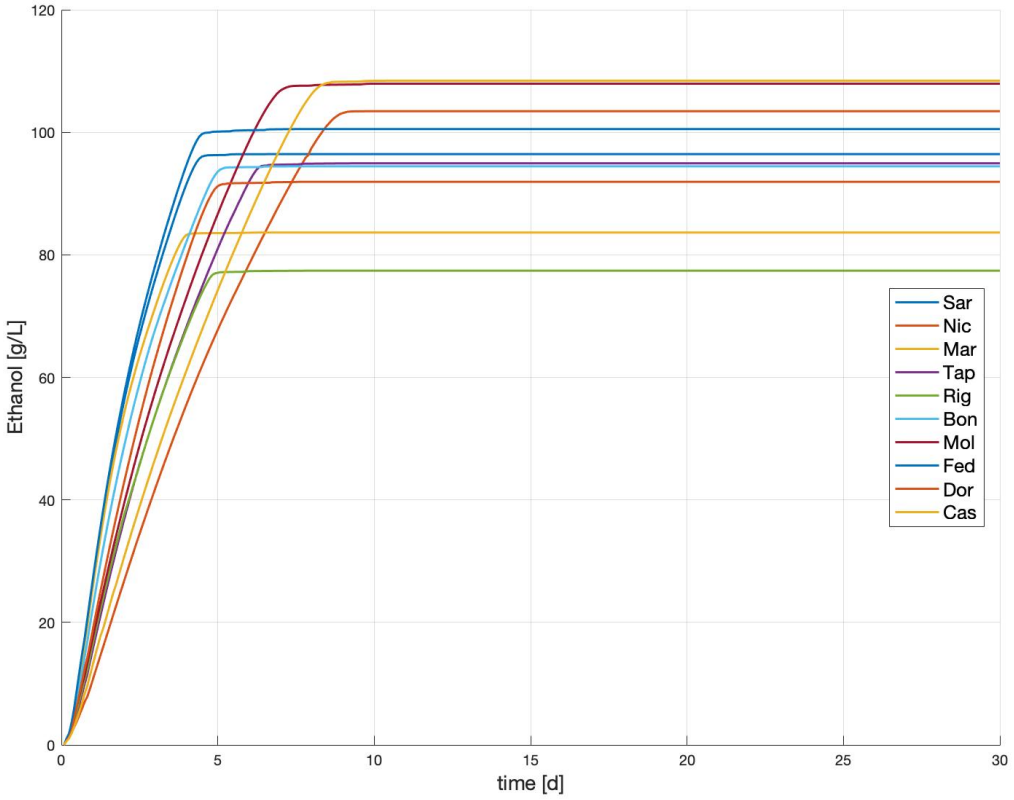


Figure 3.12: Kinetics nanovinifications - Period 1.

| Supplier *name* | $S_0$ $g/L$ | $YAN_0$ $mg/L$ | $R$ $\cdot 10^3$ | $E_{end}$ % | $t_f$ $d$ |
|---|---|---|---|---|---|
| Bon | 197,1 | 84 | 0,43 | 11.97 | 5.58 |
| Cas | 217,7 | 35 | 0,16 | 13.74 | 8.95 |
| Dor | 190,6 | 64 | 0,34 | 11.65 | 5.66 |
| Fed | 209 | 111 | 0,53 | 12.74 | 5.16 |
| Mar | 176,4 | 91 | 0,52 | 10.60 | 4.41 |
| Mol | 220,7 | 77 | 0,35 | 13.68 | 7.70 |
| Nic | 213,2 | 46 | 0,22 | 13.11 | 9.45 |
| Rig | 163,2 | 37 | 0,23 | 9.81 | 5.41 |
| Sar | 202,9 | 121 | 0,60 | 12.22 | 4.91 |
| Tap | 196,2 | 56 | 0,29 | 12.03 | 6.91 |

Table 3.4: Analyses for the first period of dehydration.

## Nanovinifications 2

In these experiments, fermentation problems have been detected in four experiments only (labelled as "Bon", "Nic", "Mol" and "Dor"). Interestingly, the latter are the experiments where the must displays the lower ratio R. Comparing "Dor" and "Bon", even if the ratio R and the YAN of the former are lower, the second one displays much slower fermentation kinetics. This could be due to the fact that, under a certain value of sugar, acceptable performances can be achieved even with low values of YAN.

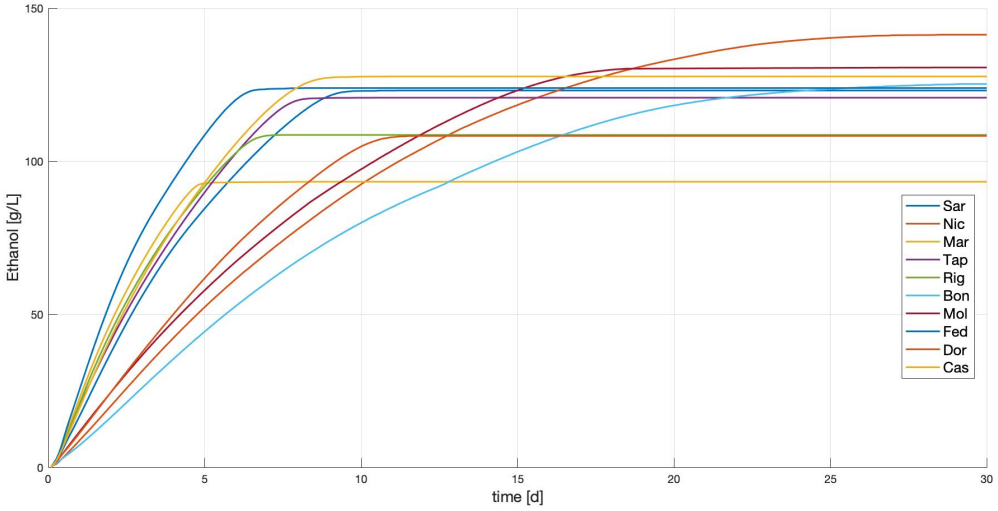


Figure 3.13: Kinetics nanovinifications - Period 2

| Supplier *name* | $S_0$ *g/L* | $YAN_0$ *mg/L* | $R$ $\cdot 10^3$ | $E_{end}$ % | $t_f$ *d* |
|---|---|---|---|---|---|
| Bon | 265,4 | 61 | 0,23 | 15.87 | 27.08 |
| Cas | 253,8 | 118 | 0,46 | 16.18 | 9.54 |
| Dor | 215,3 | 38 | 0,18 | 13.72 | 11.79 |
| Fed | 244,1 | 116 | 0,48 | 15.70 | 7.41 |
| Mar | 188,6 | 86 | 0,46 | 11.82 | 5.58 |
| Mol | 253 | 57 | 0,23 | 16.55 | 18.70 |
| Nic | 293,2 | 103 | 0,35 | 17.91 | 26.66 |
| Rig | 215,3 | 81 | 0,38 | 13.76 | 7.50 |
| Sar | 244,8 | 96 | 0,39 | 15.60 | 10.04 |
| Tap | 237,1 | 96 | 0,40 | 15.30 | 8.62 |

Table 3.5: Analyses for the second period of dehydration.

## Nanovinifications 3

In these experiments, we can observe very good fermentative performance and great similarity between the results. The initial conditions, after more that fifty days of dehydration, are close to the ones in winery. In fact, the sugar values increase and the YAN values are acceptable (in large scale vinification the YAN value is often corrected to reach values around $150 - 180 \text{mg}\,\text{L}^{-1}$ [17]. Here we can highlight the highest value of $R$ for "Fed"; despite the medium-high initial sugar concentration, its fermentation ends in a very short time.

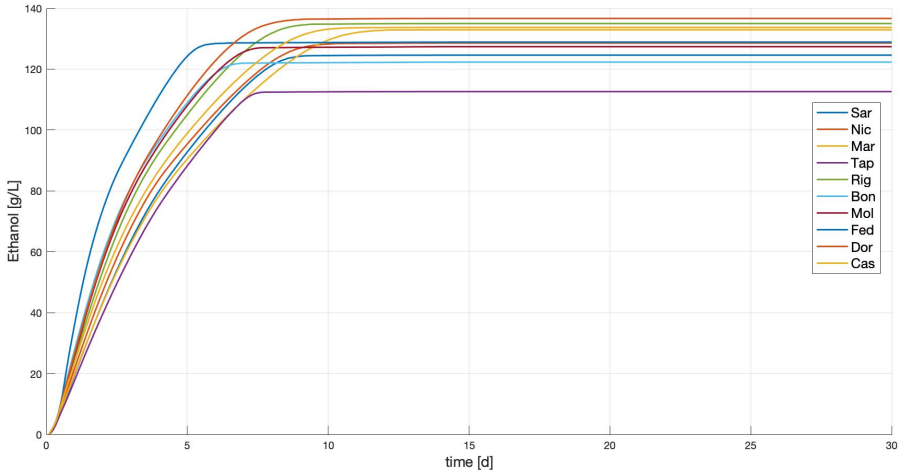


Figure 3.14: Kinetics nanovinifications - Period 3

| Supplier *name* | $S_0$ *g/L* | $\text{YAN}_0$ *mg/L* | $R$ $\cdot 10^3$ | $E_{end}$ % | $t_f$ *d* |
|---|---|---|---|---|---|
| Bon | 251,6 | 170 | 0,68 | 15.50 | 7.25 |
| Cas | 271,4 | 173 | 0,64 | 16.94 | 10.79 |
| Dor | 278,4 | 210 | 0,75 | 17.32 | 9.45 |
| Fed | 266,4 | 250 | 0,94 | 16.33 | 6.75 |
| Mar | 269,4 | 138 | 0,51 | 16.85 | 12.37 |
| Mol | 259,9 | 176 | 0,68 | 16.14 | 7.87 |
| Nic | 262,1 | 149 | 0,57 | 16.29 | 10.54 |
| Rig | 273,6 | 184 | 0,67 | 17.11 | 9.70 |
| Sar | 251,4 | 127 | 0,51 | 15.79 | 9.45 |
| Tap | 230,3 | 85 | 0,37 | 14.27 | 7.95 |

Table 3.6: Analyses for the third period of dehydration.

## Nanovinifications 4

The conditions of the must in these experiments are actually the ones detected in winery: we can appreciate the large values of the ratio $R$ and the sugar $S_0$. In view of this, the musts undergo efficient fermentations, except for "Mar": specifically, its fermentation rate (together with $t_f$) is slightly larger than in the other cases, i.e. $R = 0.47$ and $S_0$ is also high.

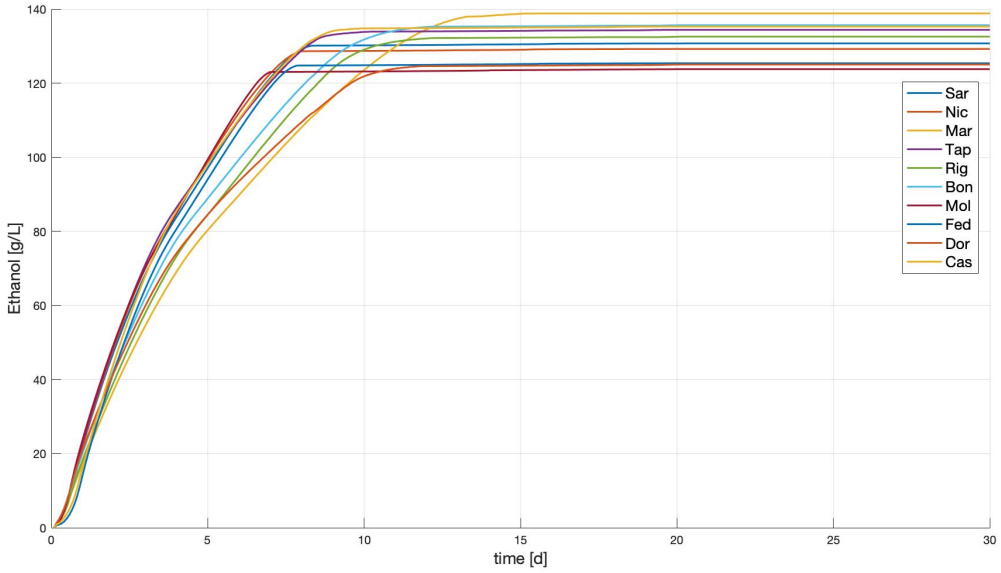


Figure 3.15: Kinetics nanovinifications - Period 4.

| Supplier *name* | $S_0$ *g/L* | $YAN_0$ *mg/L* | $R$ $\cdot 10^3$ | $E_{end}$ % | $t_f$ *d* |
|---|---|---|---|---|---|
| Bon | 279,3 | 162 | 0,58 | 17.19 | 12.29 |
| Cas | 278,5 | 194 | 0,70 | 17.14 | 10.12 |
| Dor | 258,6 | 200 | 0,77 | 15.84 | 12.04 |
| Fed | 259,8 | 187 | 0,72 | 15.89 | 8.12 |
| Mar | 285,9 | 135 | 0,47 | 17.59 | 13.54 |
| Mol | 255,4 | 200 | 0,78 | 15.69 | 7.33 |
| Nic | 269 | 210 | 0,78 | 16.38 | 8.29 |
| Rig | 267,6 | 152 | 0,57 | 16.80 | 12.37 |
| Sar | 272,3 | 210 | 0,77 | 16.57 | 8.54 |
| Tap | 276,3 | 196 | 0,71 | 17.03 | 10.45 |

Table 3.7: Analyses for the fourth period of dehydration.

## Final Comments on Nanovinifications 1-4

The data related to these nanovinifications are characterized by extreme variability. Note that the sugar and yeast assimilable nitrogen trends are not always increasing with respect to the time spent in the deyhdration phase, as shown in Figure 3.16.

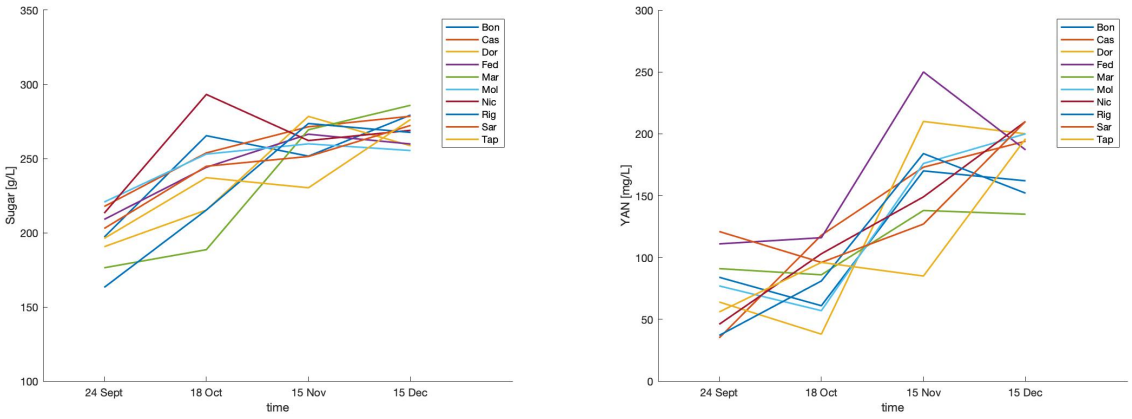


Figure 3.16: Sugar and YAN trends over the four periods.

This is due to the fact that the sampling plan occurred during the conferral, which was one-month long, and it was not possible to have control of the pallets from which grapes were sampled. Moreover, it was not possible to supervise the whole sample collection from the vintage phase. In view of this, it can be assumed that the samples were not completely consistent. This could be a problem for the study of the drying phase trends, but it is not an issue for the objectives of the thesis. Indeed, the evolution of the parameters during the drying phase has not been studied. On the contrary, the variety of the initial conditions has great importance and, as can be seen in Tables 3.4-3.7, the sugar and yeast assimilable nitrogen values are sufficiently variable.

### 3.8.2. Must Sample Experiments

#### Nanovinifications 5

In this section the results of the nanovinification experiments with the industrial must are presented, see Table 3.8. As we can see in Figure 3.17, fermentation times are considerably larger than in nanovinifications 1-4. This depends on the fact that the ratio $R$ is low: while the value of sugar is by far the highest ($330\,\mathrm{g\,L^{-1}}$), the initial YAN concentration is $104\,\mathrm{mg\,L^{-1}}$, that is one of the lowest value of our analyses. However, the initial value of sugar may be subject to a relevant measurement inaccuracy due to possible non-homogeneity of the sampled liquid. The value of $S_0$ should be around $308\,\mathrm{g\,L^{-1}}$, according to the *Sartori* winery measurement.

The nanovinifications 5 experiments can be surely considered as characterized by stuck fermentation (see Section 2.2). In fact, the kinetics proceeded very slowly. Moreover, the fermentation rate is basically equal to zero after twenty days, but the final concentration of sugar is above $30\,\mathrm{g\,L^{-1}}$. This means that the sugar is not exhausted, the production of ethanol is not finished, and there is no yeast activity that can complete the fermentative process.
Note that values of $S_{end}$ in the previous experiments (nanovinifications 1-4) are not reported because all the sugars were consumed by fermentation.

| $S_0$ | $\mathrm{YAN}_0$ | $R$ |
|---|---|---|
| $g/L$ | $mg/L$ | $\cdot 10^3$ |
| 330 | 104 | 0.32 |

| Exp n° | $E_{end}$ | $S_{end}$ | $t_f$ |
|---|---|---|---|
| - | % | g/L | $d$ |
| 1 | 18.11 | 34,6 | 19.95 |
| 2 | 17.59 | 40,7 | 20.70 |
| 3 | 17.96 | 37,5 | 19.79 |
| 4 | 17.88 | 39 | 20.70 |
| 5 | 18.25 | 32,9 | 20.79 |

Table 3.8: Analyses of the industrial must, used for nanovinification 5 and microvinification (left). Analyses of the wine obtained from nanovinification 5 (right).

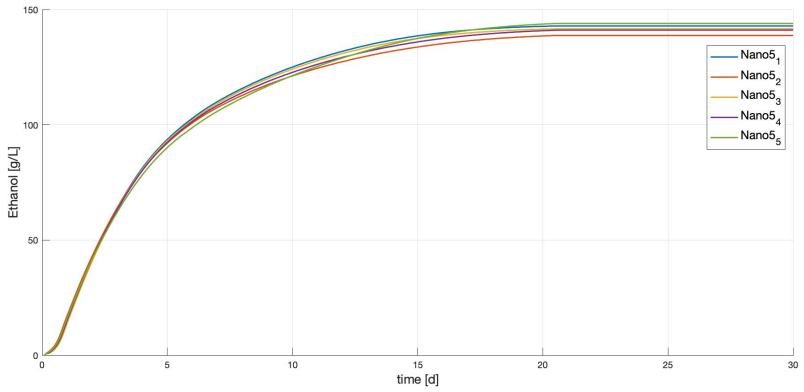


Figure 3.17: Kinetics nanovinifications - Period 5, with industrial must.

## Microvinifications

In Figure 3.18 we depict the comparison between the kinetics of the experiments carried out using the industrial must, i.e., nanovinifications 5 and microvinification. Note that the two different systems behave in the same way. This is an important result in the perspective outlined by the system project. In fact, as also confirmed by the partner *Ever - Italiana Biotecnologie*, the two processes are comparable. This correspondence has been useful for some considerations done in the optimization study (presented in Section 6.2.1).

Also in this case, the fermentation is stuck: the final value of sugar concentration is $37.19\,\mathrm{g\,L^{-1}}$, and the same considerations done for the nanovinification 5 must be done. In fact, applying the formula for the conversion from initial sugar to potential ethanol ($E_{pot} = 0.057 \cdot S_0$), we can conclude that 2.1% (corresponding to $16.72\,\mathrm{g\,L^{-1}}$) of alcohol is not developed.

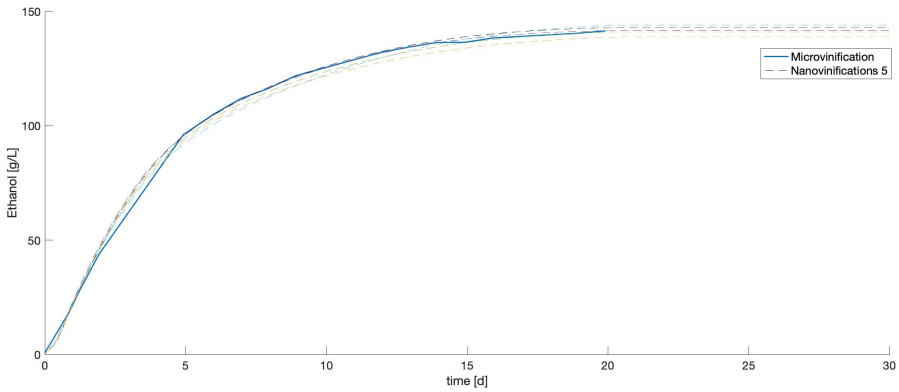


Figure 3.18: Comparison between the nanovinifications 5 (dotted lines) and the microvinification experiment (continuous line).

## 3.9. Analysis and Validation of Fermentative Performances

Some fermentation kinetics performance indicators are used in the winery in order to evaluate the progress of the fermentation and to intervene if necessary. Acceptability ranges are important to make decisions and to take actions in order to avoid, for example, stuck or sluggish fermentations. The performance indicators are:

- Maximum fermentation rate $\frac{dE}{dt}_{max}$;
- Time required to reach the maximum fermentation rate $t_{mr}$;
- Ethanol produced at the maximum fermentation rate $E(t_{mr})$;
- Latency time $t_{lag}$;
- Ethanol produced at the end of the lag phase $E(t_{lag})$;
- Duration of the stationary phase $t_s$.

These indicators are calculated from the ethanol curve. In particular, the maximum fermentation rate corresponds to the maximum slope of the tangent to the curve and is obtained by calculating the maximum value of the ethanol derivative over time. On the other hand, the intersection between the tangent and the time axis identifies the latency time. Finally, the duration of the stationary phase corresponds to the time elapsing between the instant when the curve reaches the maximum rate of fermentation and the moment when all the sugar is consumed (i.e., when the ethanol reaches its maximum value).

According to the data base of the *Ever - Italiana Biotecnologie* laboratory, the data obtained in nanovinification 4 (see Table 3.9) are in line with the industrial ones. For our case study, fermentation time intervals between 9 and 13 days are aligned with those of the *Sartori* industrial vinification (fermentation time of 15 days).

| $t_{lag}$ d | $E(t_{lag})$ $g\,L^{-1}$ | $\frac{dE}{dt}_{max}$ $g\,L^{-1}\,h^{-1}$ | $t_{mr}$ d | $E(t_{mr})$ $g\,L^{-1}$ | $t_s$ d |
|---|---|---|---|---|---|
| 0.9043 | 2.7137 | 1.3280 | 1.2816 | 11.8679 | 9.6117 |

Table 3.9: Performance indices mean values of the fourth period experiments.

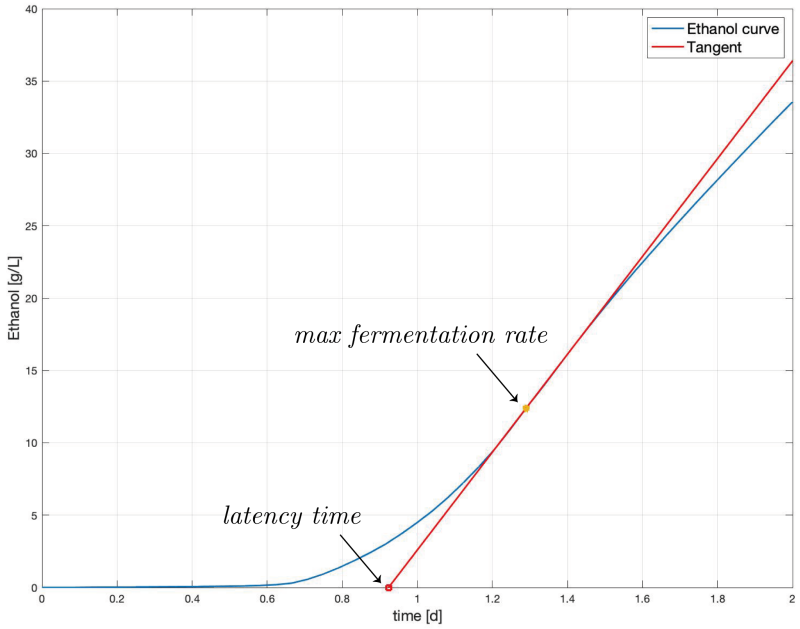


Figure 3.19: Example of latency time identification, highlighting the steepest tangent intersection with the x-axis.

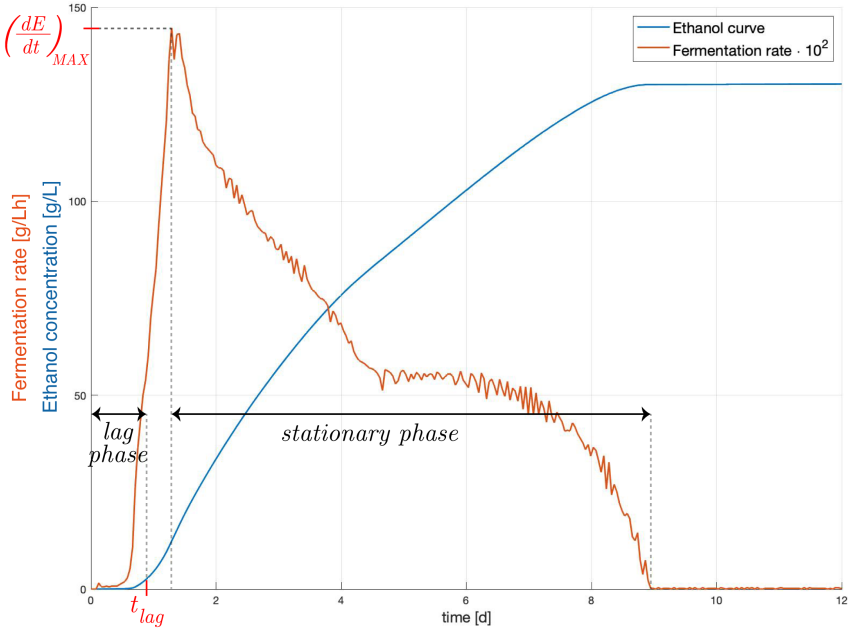


Figure 3.20: Example of lag and stationary phases identification. The maximum fermentation rate is highlighted.

# 4 | Fermentation Models Analysis

## 4.1. Introduction to Batch Processes

The term *batch process* is used to refer generically to both *batch*, where all the ingredients are fed at the beginning of the operation, and *fed-batch* ones, in which material can be added during the process [4]. In a batch process, only air/gas exchange, anti-foam or pH controlling agents can be added or removed. Winemaking processes, at industrial scale, are a fed-batch ones. In this project the analyzed models have been tested and validated with batch processes data.

Despite batch processes are simple to set up and operate, it is quite challenging to model, monitor, and control them. They are characterized by time-varying dynamics by complex, nonlinear physiological phenomena that are difficult to model. For these reasons, monitoring process modelling, variables trajectories definition, quality assessment and product safety are challenging. Models referring to this kind of processes can be classified into two groups [4]:

- First-principles (fundamental) models, that are based on fundamental physical laws, such as the conservation of mass, energy and momentum. The importance of this kind of models is that they relate key features, providing analytical expressions for the dynamic behavior of the physical system.
- Data-based (empirical, black box) models, that provide relationships between measured inputs and outputs describing how the system responds to different inputs. In this case the model development is much faster than first principles models, but less accurate, robust and general.

For this project many models have been analyzed, all first-principles ones. In the literature there are some attempts to develop data-based models of wine fermentation, but they are not very reliable. In fact, they are not validated in a sufficiently accurate way.

## 4.2. State of Art and Main Assumptions

Over the years, advances in research in many scientific fields, such as chemical process engineering, have revolutionized the understanding of the fermentation process in general, including also the winemaking process. According to the review edited by Miller et al. [13], there are three phenomena that require to be modelled:

- Well-mixed fermentation kinetics;
- Heat and mass transfer in heterogeneous wine fermenters;
- Phenolic extraction during wine fermentation.

The first one studies the growth of wine yeast (in most cases the latter consist of *Saccharomyces Cerevisiae*), the consumption of sugars and other nutrients, ethanol production, heat generation, and the increase of primary and secondary byproducts during fermentation [13]. The second one examines the differences between industrial and well-mixed fermentation in terms of creation of temperature gradients, which can be detected especially at large scale. The third one deals with phenolic extraction, that is what gives red wine its distinct color and mouthfeel: the latter is strongly dependent on temperature, ethanol concentration, and fermentation conditions. To gain a physical understanding of the process (which is necessary to optimize it under both the sustainability and the quality perspectives), an accurate quantitative modelling of these three phenomena is required. However, only the first category will be taken into account in this thesis for reasons concerning instrumentation limitations.

A well-mixed system is a system where all fluids whitin the fermentor are homogeneous. To verify this assumption, Vlassides and Block [23] measured the stratification of solids and yeast in white wine fermentation at pilot scale (1200 L) in the absence of any external agitation. When the yeast is properly hydrated, they have found that, although the system is initially heterogeneous, as yeast proliferated and fermentation proceeded, the fermentor eventually behaved as a well-mixed system. Schwinn et al. [21] also addressed this question. In the latter work, a similar experiment at multiple scale (105, 2500 and 7000 L) has been performed without agitation. The system displayed an heterogeneous behaviour until the fourth day at the 7000-liter scale, after which it could be considered as well-mixed. Lastly, measuring the temperature, sugar and ethanol concentration at different heights of the fermentor, Malherbe [10] found no heterogeneity in tanks of dimension up to 11.000 L in white wine fermentation. In view of these results, it can be assumed that a system can be considered well-mixed if its volume is smaller than 10.000 L. Unfortunately, this assumption applies only to white wine fermentation. As far as red fermentation is

concerned, due to the inclusion of pomace in the must, at essentially any scale the system exhibits a non-well-mixed behavior. During the process, substantial temperature gradients are produced and, even though pump overs and punch downs are effective at eliminating them, they rapidly reform in hours (Schmid et al [20]).

In the literature, different studies can be found on red wine fermentation modelling at industrial scale. The work of Zenteno et al. [25] provides a first attempt to quantify the evolution of temperature gradients. Also, Miller et al. [12, 14, 15] derives a higher fidelity spatial fermentation model utilizing computational fluid dynamics and finite element analysis to generate spatial and temporal reactor engineering predictions. This work was extended to include also some phenolic species extraction dynamics: this combined comprehensive model allows for the prediction of phenolic concentration and concentration gradients over a wide range of winemaking conditions and grape parameters. The major limitations of this model are two: it is computationally expensive, and it can track few phenolic species in the limited range of temperatures of 25°C-30°C. Due to his huge complexity and to his need for highly performance instrumentations, such as advanced computer processors and dedicated programs, it will not be treated in this thesis. Consequently, the study of this thesis will end with the modellization of the laboratory fermentation process.

The experiments that have been carried out could be cast as white wine vinification ones, because the fermentor volumes available were too small to allow for the inclusion of grape solids in order to simulate red wine vinification. Therefore, in the choice of the model to be used, only well-mixed models were considered. In addition to adopting this well-mixed assumption, these models treat yeast as an "unstructured" matter, ignoring the biochemical machinations inside the cells. For this reason, the predictions obtained by the models taken into consideration would be sufficient in most cases, but do not help to understand the physiology of the yeast or the impact of fermentation in the central metabolism. The work of all the considered authors, treats the cell as a "black box", consuming nutrients and releasing products into the extracellular environment. Models of "structured" or "flux-based" kind have to manage the myriad of processes within the cell. The aim of this thesis is to define a suitable model for the laboratory scale vinification. This is the base for a wider study, that aims to manage also industrial vinification and find a model that could be validated in real life winemaking conditions.

## 4.3. Description of Fermentation Kinetics

This section describes the kinetics of alcoholic fermentation in winemaking conditions [17], the main parameters that affect these kinetics, and the main elements that can be controlled to improve fermentation and modify wine properties. In a standard fermentation, performed by a pure culture of *Saccharomyces Cerevisiae* at constant temperature, three main phases are observed: *lag*, *growth*, and *stationary phases*, which are discussed in the following sections.

### Lag Phase

The lag phase is usually defined as the phase before "active fermentation". This phase corresponds to the progressive saturation of the medium in $CO_2$. A yeast cell population of approximately $10^7$ cells/ml is reached by the end of this phase, corresponding to 2–3 generations. Here, less than $4\,\mathrm{g\,L^{-1}}$ of sugar is consumed and, at the same time $2\,\mathrm{g\,L^{-1}}$ of ethanol is produced [17].

### Growth Phase

The growth phase, during which 20–40% of the sugar is consumed, lasts from the end of the lag phase until the maximum cells population is reached. More specifically, at the beginning of this phase, the yeast population increases exponentially, i.e., at a constant specific growth rate.

Considering the measure of $CO_2$, strongly linked to both the ethanol and sugar kinetics, it can be observed that:

1. The maximum specific $CO_2$ production rate is reached very soon, i.e., when the sugar concentration has reduced by less than $10\,\mathrm{g\,L^{-1}}$.
2. The maximum $CO_2$ production rate $(\frac{dCO_2}{dt})_{max}$ is reached later but always before the end of cell growth.

Usually, the growth phase ends when assimilable nitrogen in the must is exhausted. This occurs when $(dCO_2/dt)$ is maximal [17].

### Stationary Phase

The stationary phase starts when the yeast cells have reached the maximum population. Most of the sugar (between 50 and 80%) is fermented during this phase thanks to the yeast, whose population cells remain almost constant [17]. This feature is specific to the

winemaking conditions. The fermentative activity of the yeast gradually decreases during this phase, because various mechanisms that inhibit yeast growth and activity during this phase. The main inhibitor is the presence of ethanol.

## 4.4. Model Comparison and Selection

Various models, with different levels of complexity, were considered for this study. In all cases, the microbiological behavior of the cells was not analyzed, but only macro-variables were taken into account. These variables are the main actors in the fermentation process. They are ethanol, sugar, nitrogen, yeast, oxygen mainly. This section compares the available models.

### 4.4.1. Introduction to Well Mixed Models

The thesis objective is to develop a model considering laboratory vinification. This choice was due to the available equipment. As discussed in Chapter 2, the fermenters used to create a database for the fermentation are either containers of reduced capacity consisting of 0.5 L bottles or 9 L jars. For this reason, it was not possible simulate the vinifications at industrial scale, performing a fermentation with the must in contact with the pomace. This means that the models that were taken into account are *well-mixed*, so it is assumed that there is homogeneity in the must. We studied the well-mixed models in the literature and apply them to the fermentation laboratory tests.

Five models have been studied from different research groups and this section aims to summarize them and to briefly illustrate the various approaches taken to describe such a complex process. To distinguish the various models, they are addressed using the name of their authors.

### 4.4.2. Malherbe Model

Malherbe et al. [10] described the main physiological dynamics which constrain the yeast activity. This paper was the first to consider the two main parameters limiting fermentation kinetics: temperature (both in isothermal and anisothermal conditions) and assimilable nitrogen (including additions at different moments of fermentation). Its effectiveness with respect to experimental data, tested in very different conditions, emphasizes the usefulness of this model for describing the main physiological dynamics of the fermentation kinetics. In fact, the model accurately predicts the fermentation kinetics of more than 80% of a large (>100) number of experiments performed with 20 wine yeast strains, 69 musts and different fermentation conditions [10].

| **Variables** |
|---|
| $S$, $N$, $X$ |
| **Experiments** |
| >100 tests, with: |
| $S$: 200-280 $\mathrm{g\,L^{-1}}$ |
| $N$: 70-570 $\mathrm{mg\,L^{-1}}$ |
| $T$: 18-30 °C |
| **Focus** |
| Accuracy of the predictive model |

This is considered a highly reliable model in the literature. In fact, other works (e.g., David & Dochain [7], see Section 4.4.5) rely on [10]. The authors use this model to produce an experimental database, to identify their models.

The model objective is to predict the glucose consumption speed and, at the same time, the amount of produced ethanol (or $CO_2$). Some variables are involved in this process: temperature (varying within a predefined range) and yeast assimilable nitrogen (YAN), which has a major effect on the yeast activity and whose concentration really depends on must characteristics. Moreover, the effects of both initial nitrogen concentration and added nitrogen have been investigated. It is known that the fermentation rate increases with initial nitrogen concentration. Nitrogen affects both population growth and the activity of the yeast (i.e., fermentation rate). For this reason it is necessary to model the yeast growth and the average activity of a single yeast cell.

#### Equations

In this model the state variables $S$, $N$ and $X$ represent the sugar, yeast assimilable nitrogen and yeast concentrations, respectively.

$$\begin{cases} \dot{S} = -X(t)\ \nu_{ST}\ N_{ST} & (4.1a) \\ \dot{N} = -X(t)\ \nu_{N} & (4.1b) \\ \dot{X} = k_1(T)\ X(t)\ [1 - \dfrac{X(t)}{X_{max}}] & (4.1c) \end{cases}$$

The term:

$$\nu_N = \frac{k_3(T)\ N}{N + K_N + K_{NI} N E^{\alpha_N}} \tag{4.2}$$

is a global estimation of the nitrogen transport and

$$\nu_{ST} = \frac{k_2(T)\ S}{S + K_S + K_{SI} S E^{\alpha_S}} \tag{4.3}$$

represents the average activity of a single glucose transporter. The function $N_{ST}$ represents the mean number of transporters in a yeast.

In the identification of the first equation of model (4.1a), it is impossible to discriminate the number of hexose transporters, $N_{ST}$, from the numerator, $k_2(T)$, of the hexose transport function, $\nu_{ST}$. Without loss of generality, Malherbe et al. [10] assumed that a bilinear function would best model the function $N_{ST}\ k_2(T)$. The model was obtained by the least-squares estimation method for nonlinear regression models. In isothermal condition the function $N_{ST}\ k_2(T)$ takes the following form:

$$N_{ST}\ k_2(T,t) = \lambda_a \frac{N_i(t)}{X(t)} + \lambda_b T(t) + \lambda_c \frac{N_i(t)}{X(t)} T(t) + \lambda_d \tag{4.4}$$

where

$$N_i(t) = N_0 + \lambda_0 [N_{add}(t) - N(t)] \tag{4.5}$$

$N_{add}$ is the amount of nitrogen added, $\lambda_0$ is a Boolean value, it is required only if nitrogen is added.
In anisothermal condition and for the temperatures in the range 18-30 °C, $N_{ST}\ k_2(T)$ is identified as follows:

$$N_{ST}\ k_2(T,t) = \lambda_1 T_{ucd}(t) N_i(t) + \lambda_2 T N_i(t) + \lambda_3 T + \lambda_4 T_{ucd} + \lambda_5 N_i(t) + \lambda_6 \tag{4.6}$$

Considering the inclusion of the term $k_2(T)$ in the function $N_{ST}\ k_2(T)$, the Equation (4.3) becomes:

$$\nu_{ST} = \frac{\bar{k}_2\ S}{S + K_S + K_{SI} S E^{\alpha_S}} \tag{4.7}$$

where $\bar{k_2}$ is a "normalizing" parameter.

In the Equations (4.1) - (4.7), $K_S$, $K_{SI}$, $K_N$, $K_{NI}$, $\alpha_S$, $\alpha_N$ are constant describing the dynamics of the variables during the fermentation process and they can be identified using experimental data. On the contrary $\lambda_a, ..., \lambda_d$ and $\lambda_1, ..., \lambda_6$ are weights and have no physical meaning. Finally, $T_{ucd}$ represents the temperature at which the growth phase ends.

### Pro & Cons

| Pros | Cons |
|---|---|
| Validated with a large and highly reliable database, in both isothermal and anisothermal conditions | It does not "sufficiently incorporates $T$ to allow prediction of stuck/sluggish fermentation" [5] |
| | The behaviour of $E$ is not modelled, instead they are deducted by $CO_2$ kinetic |
| | "Not suitable for control purposes" [7] |

## 4.4.3. Scaglia Model

The objective of the paper [18] is to implement a predictive control algorithm to monitor and control a wide variety of wine fermentations. For example, some fermentations need to follow a specific temperature profile in order to achieve particular organoleptic properties. The model is first principles one, defined to describe the fermentation of Syrah wine in a batch bioreactor under controlled bench-scale laboratory conditions. The authors modelled the fermentation process with a set of equations which are based on the mass balances on cells, substrate as carbon source, and ethanol.
The authors based their work on experimental data provided in other two studies: Fleet [9] and Toro & Vazquez [22].

| Variables |
|---|
| $X$, $S$, $CO_2$ |
| **Experiments** |
| Fleet 1993 [9] |
| Toro & Vazquez 2003 [22] |
| **Focus** |
| Improve and adapt fermentative model of Syrah wine |

## Equations

The state variables are $X$, $S$, $CO_2$ and $P$ representing yeast and sugar concentrations, produced carbon dioxide and accumulated pressure, respectively.

$$\begin{cases} \dot{X} = \Psi(CO_2)\ A\mu_m \dfrac{S}{S + K_S B^a} X \left(1 - \dfrac{X}{A\mu_m \frac{S}{(S+K_S B^a)\beta}}\right) & (4.8a) \\ \qquad + (1 - \Psi(CO_2)) \left(CX\dfrac{dS}{dt} - DX\right) & \\ \dot{S} = \dfrac{1}{Y_{X/S}\left[-X\left(\mu_m \frac{S}{S+K_S B^b} - EX\right)\right]} - FX & (4.8b) \\ C\dot{O}_2 = G\mu_m \dfrac{S}{S + K_S B^c} X + \dfrac{d}{dt}\left(H\mu_m\ \Omega(S)X + IX\right) & (4.8c) \\ \dot{P} = \dfrac{1}{Y_{CO_2/P}} C\dot{O}_2 & (4.8d) \\ & (4.8e) \end{cases}$$

In (4.8) we used the following functions:

$$\Psi(CO_2) = \frac{e^{-CO_2 - CO_{2,95}}}{e^{CO_2 - CO_{2,95}} + e^{-CO_2 - CO_{2,95}}} \tag{4.9}$$

$$\Omega(S) = \frac{S^2}{(S + K_S B^d)(S + K_S B^e)} \tag{4.10}$$

Differently from the models presented in the other sections of this chapter, where the fermentation kinetics are described by Michaëlis-Menten equations, here the yeast growth is modeled with a variant of Verhulst logistic equation, i.e., a sigmoidal curve used to describe biological processes (see Figure 4.1), and a Monod equation (a common approach to describe enzymatic growth kinetics, see Figure 4.1).

The parameter $\mu_m$ is the maximum specific cellular growth rate and $\beta$ represents the Verlhurst's equation coefficient. The first term of (4.8a) defines if the ongoing fermentation cellular step corresponds to growth or death. The growth rate (4.11) is modeled by the Monod kinetic.

$$\mu_m \frac{S}{S + K_S B^a} \tag{4.11}$$

where $K_S$ is the substrate saturation coefficient.

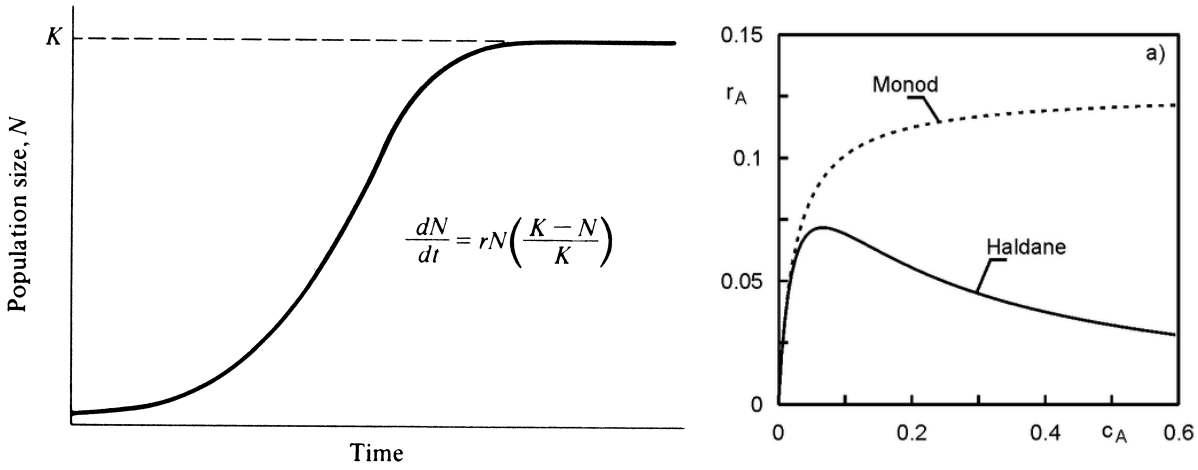


Figure 4.1: Generic Verhulst logistic kinetics and equation, where $N$ is the population, $r$ is the growth rate and $K$ is the maximum population size (left). Comparison between Monod and Haldane kinetics (right), in Section 4.4.6 further information can be found.

The death rate is modelled so as to be consistent with the experimental evidence that the faster the decrease of substrate concentration, the larger the increase in the cellular death rate, i.e.,

$$CX\frac{dS}{dt} - DX \tag{4.12}$$

The parameters $A, B, a, b, c$, etc. are identification coefficient. $Y_{X/S}$ is the rate of cells formed per consumed substrate and $Y_{CO_2/P}$ is the rate of $CO_2$ formed per produced ethanol.

## Pro & Cons

| Pros | Cons |
|---|---|
| Predict accurately the the fermentation evolution | Nitrogen effect on fermentation kinetics is not considered |
| | Complexity of the equations is considerably higher than the other models |

### 4.4.4. Coleman Model

The Coleman model [5] incorporates a model previously developed by Cramer et al. [6] with one describing the behavior of the system at varying temperatures. The aim of the author was to develop a model that could predict the course and possible problems of fermentation (sluggish and stuck) according to three main elements: the temperature, the initial nitrogen, and the sugar concentrations.

| **Variables** |
|---|
| $X$, $X_A$, $N$, $E$, $S$ |
| **Experiments** |
| several tests, with: |
| $S$: 265-300 $\mathrm{g\,L^{-1}}$ |
| $N$: 80-330 $\mathrm{mg\,L^{-1}}$ |
| $T$: 11-35 °C |
| **Focus** |
| managing stuck and slugghish fermentation |

#### Equations

The state variables are $X$, $X_A$, $N$, $E$, $S$ representing yeast, active yeast, YAN, ethanol, and sugar concentrations, respectively.

$$\begin{cases} \dot{X} &= \mu(N,T)\ X_A & (4.13a) \\ \dot{X_A} &= \mu(N,T)\ X_A\ - k_d(E,T)\ X_A & (4.13b) \\ \dot{N} &= -\dfrac{\mu(N,T)\ X_A}{Y_{X/N}} & (4.13c) \\ \dot{E} &= \beta(S,T)\ X_A & (4.13d) \\ \dot{S} &= -\dfrac{\beta(S,T)\ X_A}{Y_{E/S}} & (4.13e) \end{cases}$$

where $Y_{X/N}$ is the yield coefficient of the cell mass growth per mass of utilized nitrogen; also, $Y_{E/S}$ represent the yield coefficient of the produced ethanol per consumed sugar.

The Coleman model, togheter with the following ones, describes the fermentation kinetics using Michaëlis-Menten kinetics, that is one of the most common approach to model enzymatic processes kinetics.

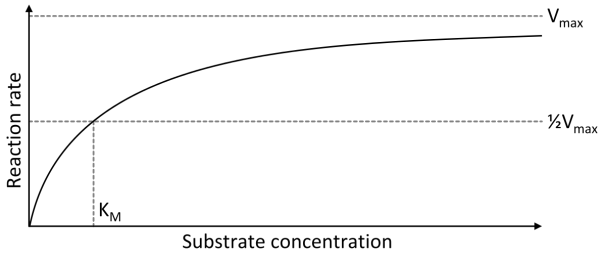


Figure 4.2: Michaëlis–Menten kinetics, with generic equation: $\dot{v} = V_{max}\frac{[S]}{K_M+[S]}$. Here, $v$ is the growth rate of the product, $V_{max}$ is the maximum rate, $K_M$ is the half saturation (or Michaëlis-Menten) constant, $[S]$ is substrate concentration.

$$\mu(N,T) = \mu_{max}(T)\ \frac{N}{K_N + N} \tag{4.14}$$

$$\beta(S,T) = \beta_{max}(T)\ \frac{S}{K_S + S} \tag{4.15}$$

$$k_d(E,T) = k'_d(T)\ E \tag{4.16}$$

Equation (4.14) represents the mortality rate of yeast cells. It can be appreciated how $X_A$ grows exactly as $X$ with the inclusion of a subtracting term representing death of yeast cells. This is modeled by $k'_d$, a death or inactivation parameter, describing the sensitivity of the cells to ethanol.
The specific growth rates $\beta(S,T)$ and $\mu(N,T)$ for the sugar and nitrogen, respectively, are described using the Michaëlis-Menten equation and $\beta_{max}(T)$ and $\mu_{max}(T)$ are the maximum specific growth rates, depending on temperature, and $Ks$, $K_N$ are the half saturation constants for $S$ and $N$ respectively.

$$\mu_{max}(T) = \mu_1 T + \mu_2 \tag{4.17}$$

$$\beta_{max}(T) = \beta_1 T + \beta_2 \tag{4.18}$$

From Equations (4.13a) and (4.13b) it can be noticed that a distinction between yeast concentration and active yeast concentration is done. Cramer et al. (11) evidenced that cells may not be completely active in terms of growth, utilization of sugar and nitrogen, and production of ethanol. Thus, $X$ will be the total concentration of yeast, while $X_A$ is only the active one.

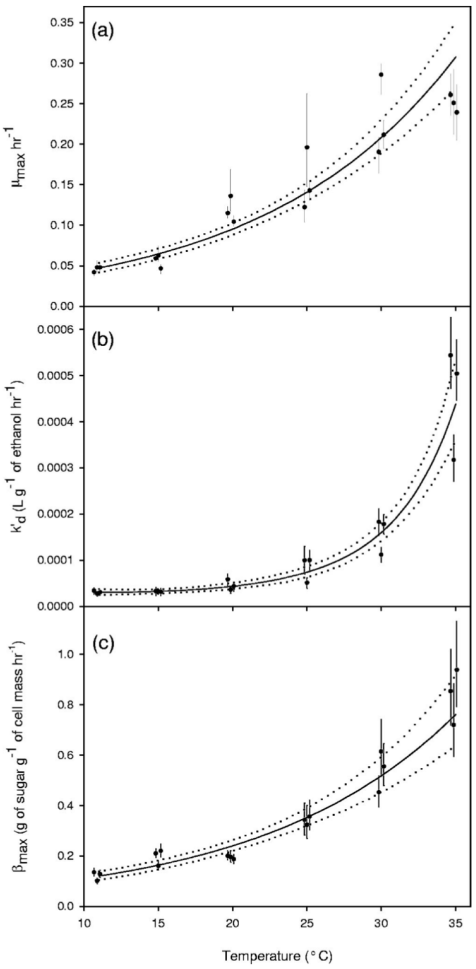


Figure 4.3: Trends for growth rates $\mu_{max}(T)$, $k'_d(T)$, $\beta_{max}(T)$ in function of Temperature, Figure from Coleman [5].

### Pro & Cons

| Pros | Cons |
|---|---|
| Validated in different initial condition of $T$, $N$, $S$. | Not validated in real winemaking conditions |
| Properly predicts also at extreme temperatures (18 and 30 ℃) | |

### 4.4.5. David & Dochain First Model

The objective of the authors was to design control tools to optimize the fermentation and at the same time obtain a determined aromatic profile. Therefore, they derived a mass-balance model based on a set of biological reactions that allow to describe the behavior of the batch fermentation process. The authors developed two formulations of the model. The first one [7] will be presented in this section and describes the fermentation with variable temperature but not for different initial nitrogen concentrations. The second formulation [7] will be presented in Section 4.4.6.

| Variables |
|---|
| $X$, $N$, $E$, $S$ |
| **Experiments** |
| Malherbe [10] experiments |
| **Focus** |
| Design control action for fermentation |

The first model, presented in [7] considers biomass $X$ growing on nitrogen $N$, which in this case is the limiting nutrient in the fermentation process. Meanwhile, sugar $S$ is enzymatically degraded into ethanol $E$ and $CO_2$, and inhibited by ethanol. The kinetics are modeled with the classical formulation of Michaëlis-Menten, as in Figure 4.2.

In this study, the authors have not run experiments for identification and validation of their predictions. Instead, they used the Malherbe model (4.1) as a simulator that provides a representative behaviour of the process. This is justified by the fact that the Malherbe model [10] is highly reliable and validated in a wide range of initial conditions and temperatures.

## Equations

The state variables for the first formulation of the David & Dochain model are $X$, $N$, $E$, and $S$ describing the yeast, YAN, ethanol, and sugar concentrations,respectively.

$$\begin{cases} \dot{X} &= \mu(N,T)\ X & (4.19a) \\ \dot{N} &= -k_1\ \mu(N,T)\ X & (4.19b) \\ \dot{E} &= C\dot{O}_2 = \ \beta(S,T)\ \dfrac{K_E(T)}{K_E(T)+E}\ X & (4.19c) \\ \dot{S} &= -k_2\ \dot{E} & (4.19d) \end{cases}$$

A great similarity with the Coleman model (4.13) should be noted. In fact, David & Dochain based their formulation on Coleman model, among others. Thus, both authors used Michaëlis-Menten kinetics, so the growth rates for nitrogen and sugar are the same equation of the previous model (4.14) and (4.15).

On the contrary, a difference can be noted in the novel term $K_E(T)$, introduced in the ethanol equation. This function represents the ethanol inhibition and it is:

$$K_E(T) = -K_{E1}T + K_{E2} \tag{4.20}$$

Also, parameters $k_1$ and $k_2$ represent the yield coefficients associated to nitrogen and sugar consumption respectively; $\mu(N,T)$ and $\beta(S,T)$ are defined in (4.14) and (4.15), where $K_N$ and $K_S$ are the half-saturation constants.

Finally, with respect to Coleman model (4.13), in this case yeast cells viability it is not considered. In fact, in Equation (4.19a) there are no terms describing the descending phase of the live cells of yeast population.

Comments about this model are postponed to the next section.

### 4.4.6. David & Dochain Second Model

The second model proposed by David & Dochain [7] is defined considering the fact that nitrogen consumption can be split into two terms: biomass growth and transport proteins synthesis. A next step [8] could be to integrate the effect of a nitrogen addition during the fermentation, but it has not been studied. During the fermentation, a portion of nitrogen is assimilated to synthesize new yeast cells, but the remaining part is mostly used in the synthesis of essential proteins. Meanwhile, the yeast assimilates glucose and nitrogen thanks to dedicated proteins called transporters.

| **Variables** |
|---|
| $X$, $N$, $Tr$, $E$, $S$ |
| **Experiments** |
| Malherbe [10] experiments |
| **Focus** |
| Design control action for fermentation |

#### Equations

The state variables $X$, $N$, $Tr$, $E$, and $S$ represent yeast, YAN, transporter proteins, ethanol, and sugar concentrations, respectively.

$$\begin{cases} \dot{X} &= \mu(N,T)\ X & (4.21a) \\ \dot{N} &= -k_1\ \mu(N,T)\ X - k_1'\ \dot{Tr} & (4.21b) \\ \dot{Tr} &= \eta_{max}(T)\ N\ X & (4.21c) \\ \dot{E} &= \beta(S,T)\dfrac{K_E(T)}{K_E(T)+E}\ X & (4.21d) \\ \dot{S} &= -k_2\ \dot{E} & (4.21e) \end{cases}$$

Basically, the formulation is very similar to the first model (4.19) with the main differences in the dynamics of nitrogen, as explained above. In this second model, David & Dochain abandoned the Michaëlis-Menten kinetics. In fact, the best curves fitting for the biomass growth (and therefore, for the $N$ dynamics) correspons with an Haldane kinetics (a more generic formulation for describing enzymatic processes), hence:

$$\mu(N,T) = \mu_{max}\ \frac{N}{K_N + N + \frac{N^2}{K_I(T)}} \tag{4.22}$$

From David & Dochain [7], experiments have shown that if the initial nitrogen concentration is low, the yeast is mainly focused on increasing the population. On the contrary, when $N_0$ is larger, the cells production increases but, meanwhile, a bigger part of nitrogen is used for the synthesis of transporters. Figure 4.4 presents the different trends for $\mu$ and

$\eta$ with different intial conditions of nitrogen:

- When $N(0)$ has a low value, $\mu$ is larger than $\eta$ and therefore $X$ grows by consuming quickly almost all the nitrogen.
- When $N(0)$ has a large value, $\eta$ is larger than $\mu$ and $Tr$ consumes quickly nitrogen when compared to $X$.

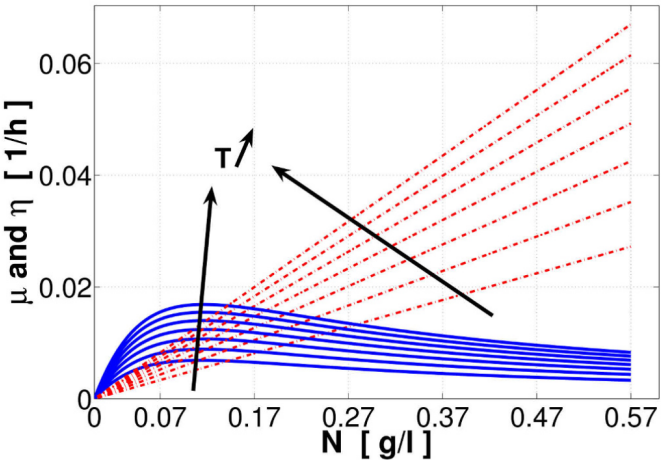


Figure 4.4: Trends for $\eta(N,T)$ and $\mu(N,T)$ with respect to the nitrogen initial condition, Figure from [7].

The functions $\eta(N,T) = \eta_{max}(T) \cdot N$, $\beta_{max}(T)$ (Equation (4.18)), and $\mu_{max}(T)$ (Equation (4.17)) are the maximum specific growth rates. It can be remarked that, in nitrogen dynamics (4.21b), the presence of the transporter inhibits the effect that nitrogen has on ethanol. This inhibition is modeled by $\eta_{max}$ which is the maximum specific reaction rate for transporter proteins and by $k_1'$, the yield coefficient associated to $Tr$ synthesis.

$K_N$ and $K_I$(T) are the half saturation constants and the inhibition constant of the Haldane law. Finally, $K_S$ is the Michaëlis-Menten constants, $K_E(T)$ represents the ethanol inhibition.

## Pro & Cons

| Pros | Cons |
|---|---|
| Unique model considering transporter proteins synthesis | Identified and validated with a simulator and not with experimental data |
| More accuracy in yeast and nitrogen behaviours | |

### 4.4.7. Schenk & Schulz Model

The Schenk & Schulz model [19] is a modified version of the Borzì model [3], which in turn is an evolution of the first David & Dochain model (4.19). The improvements that Borzì has brought are the inclusion of the oxygen dynamic and the yeast dying phase term. Afterwards, Schenk & Schulz improved the formulation of both this aspects. According to Borzì, yeast activity is equal to zero in absence of oxygen. In alcoholic fermentation, even in the absence of oxygen, yeast is not inactive, therefore, an adjustment in the oxygen equation was needed. Moreover, the yeast cells death rate is designed in a different way with respect to the Borzì model. More explanations about oxygen and death rate follow in the next sections.

| **Variables** |
| --- |
| $X$, $N$, $E$, $S$, $O_2$ |
| **Experiments** |
| Experiments appliyng control action on Riesling must |
| **Focus** |
| Design control action with EMPC |

In addition, for temperature control purposes, an equation describing the temperature development was included. In fact, the purposes of the article are: minimizing the energy consumption and maintaining the quality of the wine invariated.

#### Equations

The model state variables $X$, $N$, $E$, $S$, and $O_2$ represent yeast, yeast assimilable nitrogen, ethanol, sugar and oxygen concentrations, respectively.

$$\begin{cases} \dot{X} &= \mu(N,T)\dfrac{S}{K_{S1}+S}\left(\dfrac{O_2}{K_O+O_2}+\epsilon\right)X - k_d X - \phi(E)\ X & \text{(4.23a)} \\ \dot{N} &= -k_1\ \mu(N,T)\dfrac{S}{K_{S1}+S}\left(\dfrac{O_2}{K_O+O_2}+\epsilon\right)X & \text{(4.23b)} \\ \dot{E} &= \beta_{max}\dfrac{S}{K_{S2}+S}\dfrac{K_E(T)}{K_E(T)+E}\ X & \text{(4.23c)} \\ \dot{S} &= -k_2\ \dot{E} - k_3\ \mu(N,T)\dfrac{S}{K_{S1}+S}\left(\dfrac{O_2}{K_O+O_2}+\epsilon\right)X & \text{(4.23d)} \\ \dot{O}_2 &= -k_4\ \mu(N,T)\dfrac{S}{K_{S1}+S}\dfrac{O_2}{K_O+O_2}\ X & \text{(4.23e)} \end{cases}$$

Being an evolution of the David & Dochain first model (4.19), the main structure of (4.23) is the same as (4.19). In fact, the kinetics are described by Michaëlis-Menten equations and the term $\mu(N,T)$ still appears, as in (4.22).

The growth of yeast is dependent on the consumption of nitrogen, sugar and oxygen. Sugar is converted into ethanol and the latter has inhibitory effect on the sugar itself.

Still, sugar has two functions: producing ethanol and serving as nutrient for yeast.

Borzì [3] was the first one that introduced the oxygen dynamics in the fermentation model. This is an improvement also in perspective of any confrontation with winery data, because the oxygen has major effect on vinification at industrial scale, as explained in Section 2.2. Moreover, Schenk & Schulz added the term $\epsilon$ to the Equation (4.23e), because even in the absence of oxygen, yeast is not totally inactive.

Another feature introduced by Borzì and integrated in the Schenk & Schulz model is the yeast cells death:

$$\phi(E) = \left(0.5 + \frac{1}{\pi} arctan(k_{d1}(E - tol))\right) k_{d2}(E - tol)^2 \tag{4.24}$$

where $tol$ is the tolerated ethanol concentration, and $k_{d1}$ 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 yeast cells undergo a death phase after the stationary one. Other phenomenon, that can cause the death of yeast cells, is described by the term $k_d \cdot E$ in Equation (4.23b).

The Michaëlis-Menten kinetics are used in the formulation of this model. Here, $\mu_{max}$ and $\beta_{max}$ are the specific grow rates and are linearly dependent on the temperature, as in previous models, Equation (4.17).

$K_N$ 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. The temperature has the following dynamics:

$$\dot{T} = \alpha_1 \, \dot{E} - \alpha_2 \, \dot{O}_2 - \alpha_3 \, (T - u_c) \, \omega_1(t) - \alpha_4 \, (T - T_{ext}) \tag{4.25}$$

In order to manipulate the temperature, the tanks available to the Schenk & Schulz team could be cooled by means of a cooling fluid flowing through a cooling element surrounding the tank. The main assumption is that, with the accumulation of ethanol, the temperature increases. This increment has a higher impact at the beginning of the fermentation where oxygen is still present. The coefficient $\alpha_1$ indicates how much heat is generated by the conversion of sugar into ethanol, the coefficient $\alpha_2$ quantifies the fact that the consumption of the oxygen reduces the accumulation of heat, while the coefficients $\alpha_3$ and $\alpha_4$ are two heat transfer coefficients. Temperature $T$ is the temperature of the tank,

$u_c$ is the temperature of the cooling fluid, which is constant, $T_{ext}$ is the temperature of the room and $\omega_1$ is the control input i.e, the cooling fluid flowrate.

### Pro & Cons

| Pros | Cons |
|---|---|
| Unique model considering death of yeast cells with good accuracy | Experimental conditions slightly different from ours |
| Introduction of $O_2$ dynamics | Slightly higher formulation complexity |
| Control action already applied with good results | |

## 4.4.8. Model Selection

This section aims to summarize the Pro & Cons of the studied models and highlights their differences. Finally, one model will be selected for our study, among the ones analyzed above.

**Malherbe** The model predicts the fermentation evolution with a good accuracy: in fact, the data bank from the experiments that Malherbe et al. have run is very large and highly reliable. They worked with different initial conditions, varying three variables: temperature from 18 °C to 30 °C, nitrogen (YAN) from 70 mg $L^{-1}$ to 570 mg $L^{-1}$, and sugar from 200 g $L^{-1}$ to 280 g $L^{-1}$. However, issue with this formulation is that the ethanol dynamics is not accurately modeled. In fact, the behaviour of ethanol is only described by its link with $CO_2$:

$$\begin{cases} S(t) = S(0) - 2.17CO_2(t) & \text{(4.26a)} \\ E(t) = 0.464(S(0) - S(t)) & \text{(4.26b)} \end{cases}$$

while in the other publications the kinetics for $E$ is more precisely designed.

Finally, according to David & Dochain [7], the Malherbe model does not consider consistent mass balances and is not simple to manipulate due to the complexity of the equations. Moreover, the large number of parameters and initial conditions used as parameters, does not allow a straightforward identification. For all of this reasons the Malherbe model has been discarded.

**Scaglia** The Scaglia model (4.8), despite the absence of nitrogen source, has proven to be satisfactory for what concerns the substrate trajectory and the performances regarding

the tracking errors. For this reasons, according to the authors, the application of this model for the the design of suitable controllers of the fermentation dynamics is promising. However, the effect of nitrogen should not be neglected, both in the light of the discussion in Section 2.1.2 and in view of possible considerations of the case of modelling the nitrogen addition effect during fermentation (that is a common procedure in winemaking), not considered in this study.
In addition, ethanol was not introduced directly into the equations governing the other variables. Also the ethanol concentration was expressed as a function of the amount of carbon dioxide. The latter is not a strong limitation, but a model that describes directly the ethanol effect and dynamics is preferable.

**Coleman** This model is the first one that can accurately predict the transformation from sluggish to normal to stuck fermentation. Also, the model is the first to accurately predict fermentation behaviours at extreme conditions (from 11 to 35 °C). Therefore, this formulation presents both the accuracy and the formulation simplicity that we are looking for in this work. However, the model selection did not fall into this model mainly because this model was further improved by David & Dochain. In fact, the ethanol dynamic is better described by the David & Dochain model (4.19), thanks to the inclusion of the term $K_E(T)$.
Another reason for discarding this model was that it have not been validated in real winemaking conditions [7].

**David & Dochain and Schenk & Schulz** David & Dochain first model (4.19) was not selected basically because it was improved firstly by Borzì, then by Schenk & Schulz. In fact, as highlighted in Section 4.4.7, the Schenk & Schulz formulation has improved this model, under various aspects. The oxygen dynamics is included, and a better understanding of the yeast cells death rate is modelled. Also, the interdependencies among the variables are more accurately modelled.

For what concerns the second model of David & Dochain (4.21), there are definitely some improvements with respect to the first model (4.19), but some limitations came out. As a matter of fact, modelling the synthesis of transporter proteins is an addition that surely improves the accuracy of the modelling, but, according to David & Dochain [7], without measurament on $Tr$ (not available neither to us, neither to David & Dochain) the variable $k_1'$ remains undetermined. Moreover, we decided to rely on models validated with real-life experiments, instead, as discussed this model has been identified and validated only using a simulated database provided by the Malherbe model (4.1).
To conclude, this second model has been taken up and improved over years by David

& Dochain themselves, focusing on the boost and shorten effect in the process duration generated by the addition of nitrogen during fermentation. Later, the modelling of the aromatic profile was also added, increasing its complexity. This model was not used because the experimentation of *Ever - Italiana Biotecnologie* did not allow the addition of nitrogen on course and the monitoring of the aroma profile. However, these models are very valid and could be investigated in the future.

To conclude, the Schenk & Schulz model was chosen to model the experimental fermentation conducted in the laboratory of *Ever - Italiana Biotecnologie*. It seems the better tradeoff between accuracy and complexity, among all the analyzed models. Moreover, it has been devised for control design purpose, that could be an interesting future perspective. Inevitably, also for this formulation, some limitations are present. For example, the experiments on which the model has been identified and validated are quite different from our setup, presented in Chapter 3. In fact, their wine had different organoleptic characteristics with respect to *Amarone*: it have $18\,\mathrm{g\,L^{-1}}$ of residual sugar, and so the fermentation is tuned to end at that sugar level. However, this problem is not so restrictive, indeed, it is enough to make some considerations to avoid it, see Chapter 5. Also, the inclusion of the oxygen dynamics is not so important for model accuracy, but it can be interesting for possible future other studies that could consider industrial-scale vinifications.

# 5 | Parameter Identification and Validation

In this section, model parameters are considered and identified. Since the chosen model has a significant number of parameters, the first problem consisted in the selection of the most suitable ones to identify and which were the ones whose literature values were to be considerable as trustable. Considering model (4.23):

- $K_N$ and $K_O$ are the Michaëlis-Menten half-saturation constants associated to nitrogen and oxygen, respectively.
- $k_1$ and $k_4$ are the yield coefficients of nitrogen and oxygen, respectively.
- $K_{S1}$ and $K_{S2}$ are two saturation constants associated to sugar. Specifically, $K_{S1}$ represents the saturation constant associated to the portion of sugar that is a nutrient for the yeast and $K_{S2}$ is the saturation constant associated to the portion of sugar necessary for the metabolization into alcohol.
- $k_2$ and $k_3$ are the yield coefficients of sugar. The first one is associated to the part of sugar converted into alcohol and the second one is related to the part of sugar which is used as a nutrient for the yeast.
- $K_E$(T) is a function dependent of the temperature, i.e.,

$$K_E(T) = -K_{E1}T + K_{E2} \tag{5.1}$$

  and has direct effect on the ethanol kinetics.

- $k_{d1}$, $k_{d2}$ and $tol$ are parameters that allow to model the function $\phi(E)$ in (4.24), representing the yeast cells death rate. The parameters $k_{d1}$ and $k_{d2}$ are associated with the death of yeast cells due to exceedance of ethanol concentration over the quantity $tol$. On the other hand, $k_d$ represents the death rate of yeast cells for other circumstances.

- $\epsilon$ allow to represent the fact that nutrients are consumed by the yeast for its activity even when there is no oxygen available.
- $\mu_{max}$ and $\beta_{max}$ are growth rates for ethanol and oxygen respectively, i.e.,

$$\mu_{max}(T) = \mu_1 T + \mu_2 \tag{5.2}$$

$$\beta_{max} = \beta_1 T + \beta_2 \tag{5.3}$$

  They are functions of temperature depending on parameters, $\mu_1$, $\mu_2$ and $\beta_1$, $\beta_2$, respectively.

Schenk & Schulz used a direct multiple shooting approach for the discretization of the parameter estimation problem. A sequential quadratic programming method was applied to minimize the objective function, consisting of the sum of squares of the weighted residuals represented by the estimating function. In [19], not all the parameters described were estimated: some of them were considered drawn from the literature. In Tables 5.1 and 5.2 the values of the estimated and the fixed ones are shown.

| Parameter | Value |
|---|---|
| $K_N$ | 0.1156 |
| $k_1$ | 0.0536 |
| $K_{S2}$ | 4.3262 |
| $K_{E1}$ | 0.2616 |
| $K_{E2}$ | 38.90 |
| $k_{d1}$ | 99.86 |
| $k_{d2}$ | 0.0021 |
| $K_O$ | 0.0007 |
| $k_4$ | 0.0025 |
| $\epsilon$ | 0.02 |
| $k_d$ | 0.01 |
| $tol$ | 79.0 |

Table 5.1: Parameters from literature.

| Param. | Initial val. | Estim. |
|---|---|---|
| $\mu_1$ | 0.08 | 0.514 |
| $\mu_2$ | 0.1858 | 4.9325 |
| $K_{S1}$ | 33.35 | 34.2695 |
| $\beta_1$ | 0.3371 | 0.3954 |
| $\beta_2$ | 0.0285 | 0.0 |
| $k_2$ | 1.2 | 1.5324 |
| $k_3$ | 15 | 15.75 |

Table 5.2: Parameters estimated.

The parameters estimated by Schenk & Schulz were, however, not suitable for our thesis, since their data and tests are based on different experiments and wine. For these reasons, we performed the parameter estimation using new data, see Section 5.3.

## 5.1. Choice of the Parameters to Estimate

The choice of the parameters to estimate was similar to the one considered by Schenk & Schulz: some were taken from the literature, while some were subject to identification. This choice was made since the large number of parameters considerably increased the computational cost and, since the ethanol profile was the only available, we attempted to avoid identifiability problems. The selected parameters to be estimated and their respective literature nominal values are shown in Table 5.3.

| Parameter | Initial value |
|---|---|
| $\mu_1$ | 0.08 |
| $\beta_1$ | 0.3371 |
| $K_{S1}$ | 33.35 |
| $k_2$ | 1.2 |
| $k_3$ | 15 |
| $K_{S2}$ | 4.3262 |
| $K_N$ | 0.1156 |
| $tol$ | - |
| $k_d$ | 0.01 |

Table 5.3: Selected parameters to be estimated.

There are small differences and several additions with respect to the choice made by Schenk & Schulz, i.e.,

- $\mu_1$ and $\beta_1$ - Working at a constant temperature of 24 °C, $\mu_{max}$ and $\beta_{max}$ in (5.2) and (5.3) assume constant values. Therefore, $\mu_1$ and $\mu_2$ are not practically identifiable when the system is not excited enough in terms of temperature. We decided to identify $\mu_1$ and draw the value of $\mu_2$ from the literature, since the latter has a minor effect on $\mu_{max}$. The same consideration has been done for $\beta_{max}$: for the same reason, only $\beta_1$ was estimated.
- $tol$ - In the simulations, we observed that the level of final ethanol was far too low with respect to the nominal value, especially when the initial concentration of sugar was higher than $210\,\mathrm{g\,L^{-1}}$. This was due to the fact that parameter $tol$ in the death yeast cells equation (4.24) was not correctly tuned. For example, considering a potential value of ethanol of $120\,\mathrm{g\,L^{-1}}$ (corresponding to about $250\,\mathrm{g\,L^{-1}}$ of sugar), the fixed value of $tol = 79\,\mathrm{g\,L^{-1}}$ starts the yeast cells death too early, preventing the

concentration of ethanol to reach its correct final value. This effect has an influence also on the sugar kinetics since the sugar could not be exhausted completely. In contrast to the other parameters, we decided to estimate *tol* through an ad-hoc formula, as discussed later.

- $K_{S2}$ - In preliminary identification attempts, despite the addition of *tol* to the parameters to be estimated, the sugar did not reach the final zero value. Therefore, in addition to $K_{S1}$, we decided to estimate the other parameter directly involved in the dynamics of sugar, that is $K_{S2}$.
- $K_N$ - to help the parameter estimation problem to find the optimal solution, $K_N$ was also added to the set of estimated parameters. This parameter has effect on almost all the state variables of (4.23); thereby, it seemed reasonable to identify it since it affects basically all the kinetics.
- $k_d$ - This parameter was estimated only for the nanovinification 5 and the microvinification tests. We decided to add it because the extreme initial conditions of the industrial must sample (high sugar, low YAN) could not permit a satisfactory fermentation prediction. Since these fermentations resulted sluggish and stuck, we assumed that it could have been useful to estimate another parameter involved in the yeast cells death.

The parameters nominal values, used as initial conditions in the iterative identification process, were chosen from [19]. An exception was made for *tol*, being it highly dependent on the sugar initial condition and on the ethanol concentration final value. In view of this, we decided to estimate it as 90% of the final ethanol concentration (for each experiment):

$$tol = E_{end} \cdot 0.9 \tag{5.4}$$

This decision was taken for two reasons:

1. The value of $79\,\mathrm{g\,L^{-1}}$ considered by Schenk & Schulz was about the 80% of the final ethanol value and their goal was not to consume all the sugar. Therefore, we assumed that increasing this percentage would have made sure that the final value of sugar would have reached $0\,\mathrm{g\,L^{-1}}$.
2. Given the great variability of initial sugar conditions among all musts, it is not possible to find a single value of *tol* that would be suitable for each operating condition.

## 5.2. Computational Procedure

The Parameter Estimator application of Simulink has been used for identification purposes. The software formulates the parameter estimation problem as an optimization one and the solution consists of the estimated parameter values set. To formulate the latter prolem we need to specify the following:

- $x$ — Design variables. They are the model parameters and initial states to be estimated.
- $F(x)$ — Objective function, also called cost function or estimation error. It is a function that calculates a measure of the difference between the simulated and measured outputs.
- $\underline{x} \leq x \leq \overline{x}$ — Bounds. They are limits on the estimated parameter values.
- $C(x)$ — Constraint function. It is a function that specifies further (nonlinear) restrictions on the design variables.

The software estimates the model parameters so as to obtain a simulated output ($y_{sim}$) that follows the measured output ($y_{meas}$). To do so, the solver minimizes the estimation error which is defined as the measure of the difference between the simulated and measured outputs and has the following form:

$$e(t) = y_{meas}(t) - y_{sim}(t)$$

The software provides two types of cost functions to process $e(t)$. The default option is the "Sum Squared Error" cost function, and the other is the "Sum Absolute Error". After various tests, better performances were observed with the first option. The cost function is therefore:

$$F(x) = \sum_{t=0}^{t_N} e(t)^2$$

where $N$ is the number of samples.

Various optimization methods are also proposed. For the case of parameter estimation, the Nonlinear Least Squares optimization method was recommended [11].

The measured ethanol data (called "experiments" in Simulink) from the various winemaking tests were uploaded together with their relative initial states, see Figure 5.1. Moreover,

the final condition of the sugar concentration has been set to $0\,\mathrm{g\,L^{-1}}$, at the corresponding time instant in which the fermentation is considered terminated. The computation of this time instant is explained in Section 3.6. Then, we have selected which parameters had to be estimated and defined the corresponding bounds, as in Figure 5.2.

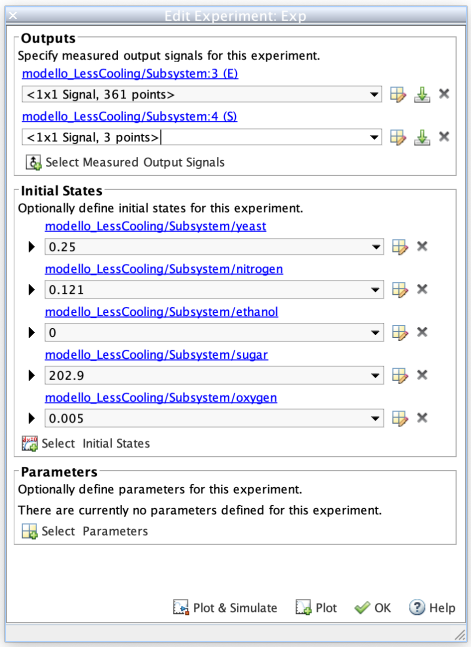


Figure 5.1: Experiments uploading on Parameters Estimator App.

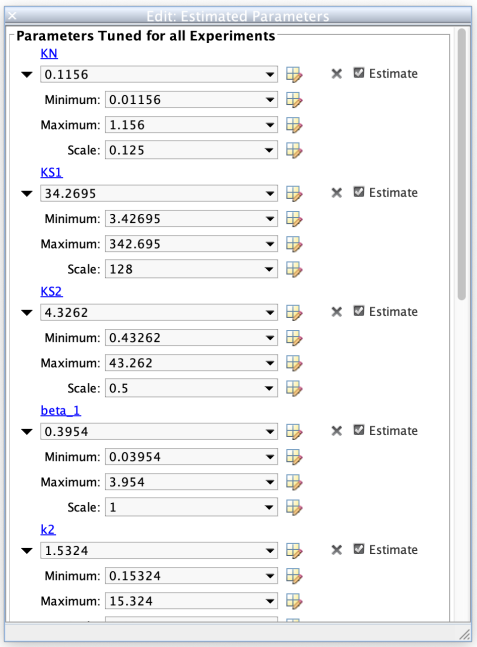


Figure 5.2: Parameters selection and their boundaries.

The application allows to choose which experiments must be used for estimation, and which for validation. To improve the model identification, we constrained the initial ($S_0$) and final condition ($S_{end} = 0$) of sugar concentration.

## 5.3. Estimation Strategies

### 5.3.1. Nanovinifications 1-4

The mathematical model defined using the data related to nanovinifications 1-4 has been devised in order to validate the chosen modelling choices. In particular we will considered such choices validated if the identified model is capable of predict the fermentation kinetics with good accuracy in different initial conditions, with special attention to the one detected in the winery.

The strategy adopted for the model identification consists of estimating the parameters over the ten experiments of the fourth drying period (Section 3.3). We decided to perform this identification since the initial conditions of these experiments were the most similar to the real winemaking conditions, in terms of initial sugar and yeast assimilable nitrogen. As a matter of fact, as explained in Section 3.8, the fourth period musts are the ones with higher $S_0$ and $\text{YAN}_0$.

The results of this strategy are presented in Table 5, that shows the initial values and the estimates. Except for the value of $\mu_1$, which changes of two orders of magnitude, a slight alteration can be noticed in all the other parameters.

| Parameter | Initial Value | Estimate |
|---|---|---|
| $\mu_1$ | 0.08 | 2.1524 |
| $\beta_1$ | 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 5.4: Parameters estimates.

From now on, we will refer to the experiments with a three letters abbreviation (reminding of the supplier's name) and a number (which stands for the dehydration period).

The identification can accurately predict the fermentation performance of both ethanol and sugar kinetics for all the ten considered experiments, with a small accuracy loss in some of them (in Figure 5.3 two examples can be observed). We assumed that the predicting difficulties could come from the ratio $R = \text{YAN}_0/S_0$. In fact, results related

to the experiments with $R < 0.6$ are less accurate than the others (only three ratios are below this threshold in these experiments, the lowest is 0.47). This is totally reasonable since a too low $R$ value corresponds to an excessive amount of sugar to be consumed with little yeast assimilable nitrogen. For this reason, the relationship between the predictive performance result and $R$ (as well as $S_0$ and $YAN$ values) must be taken into account.

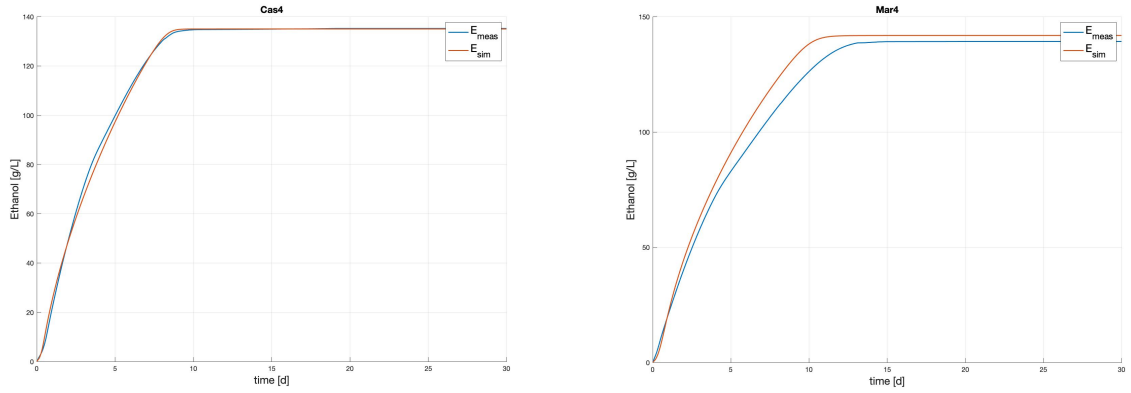


Figure 5.3: Best (Cas4) and worst (Mar4) predictions between the ten considered experiments. Their $R$ values are 0.7 and 0.47, respectively.

Given the good performances of the model on the fourth period tests, we have decided to validate it on the other periods experiments. We have compared the experimental kinetics with the simulated ones and observed the differences. Among the other thirty considered experiments, eight were considered outliers:

- **Cas1 Nic1 Rig1 Dor2 Mol2 Bon2** - All these experiments have $R < 0.25$ and are characterized by very troubling fermentations (see sluggish fermentation, see Chapter 2).
- **Nic2** - Despite its $R > 0.25$, this experiment experienced severe fermentation troubles; its process lasted almost 25 days (three times with respect to the others). Even if it has an initial condition of $\mathrm{YAN}_0 = 103\,\mathrm{mg\,L^{-1}}$ (which is an average value), we assumed that the value of initial sugar $S_0 = 293.2\,\mathrm{g\,L^{-1}}$ (the highest in all the four periods) does not allow good fermentation performances.
- **Fed3** - Its value $R = 0.93$ is far from the other $R$ values. In fact, it is the only $R$ above 0.78 and it has the highest value in YAN, $250\,\mathrm{mg\,L^{-1}}$. This experiment experienced the fastest fermentation rate, even with a considerable initial value of sugar $S_0 = 266.4\,\mathrm{g\,L^{-1}}$.

Considering this outliers-removal, a good portion of experiments were fitted with good accuracy. In the scatter plot (Figure 5.4) it is possible to notice which experiments (defined by their initial conditions) were "well predicted" and which were not, highlighting the outliers.

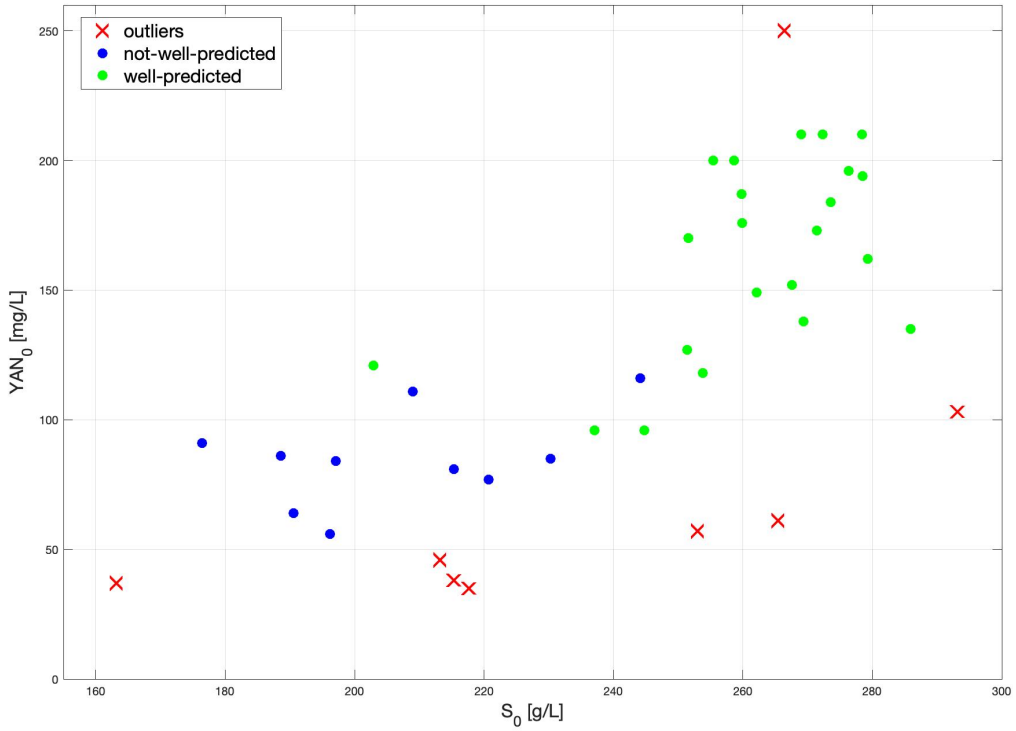


Figure 5.4: "Well predicted" experiments together with highlightened outliers.

Some examples of the validation procedure can be observed in Figure 5.5. Since we only had this measurement available, ethanol kinetics was considered in validation phase.

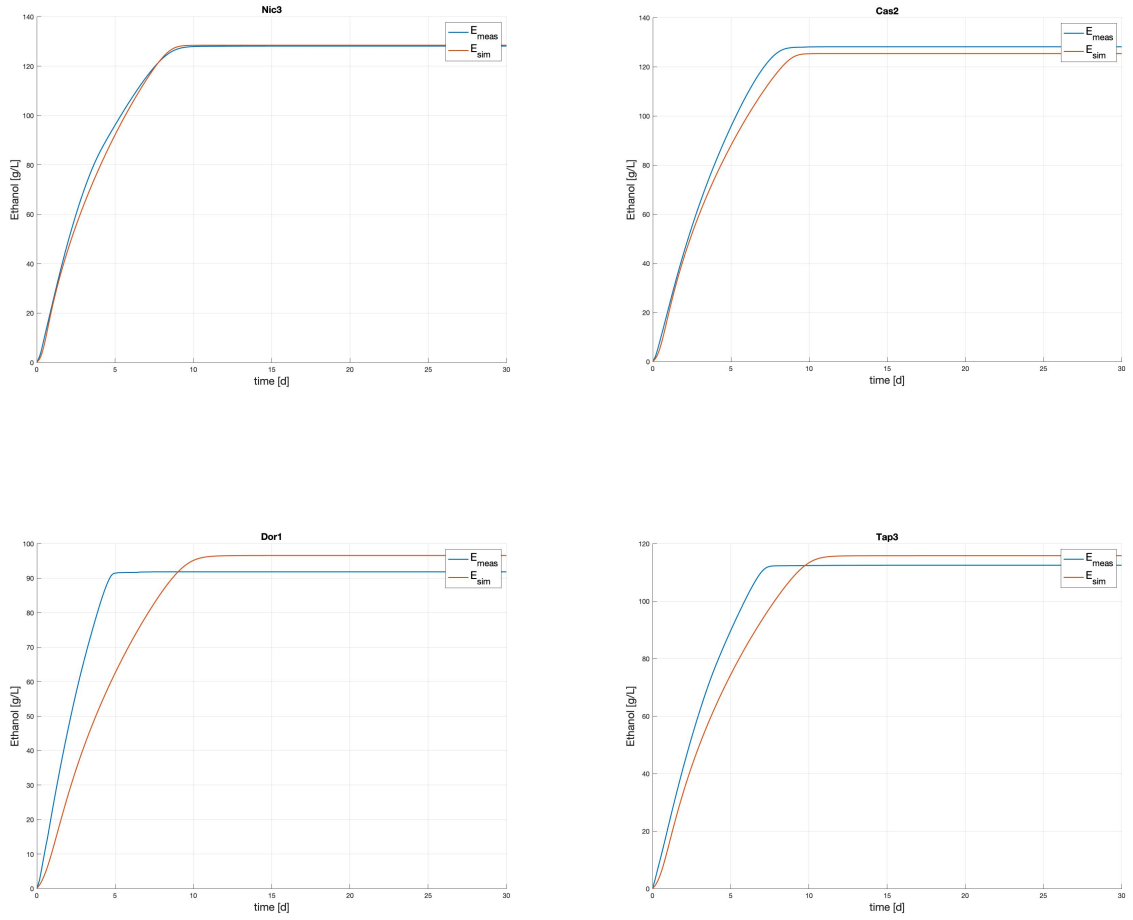


Figure 5.5: "Good" and "bad" predictions on various periods experiments. Nic3 and Cas2 are two "well" predicted experiments: their $R$ values are 0.57 and 0.46, respectively. Dor1 and Tap3 are "bad" predicted experiments: their $R$ values are 0.34 and 0.37, respectively.

The prediction performances of the model are evaluated in terms of:

1. Fermentation time;
2. Final ethanol concentration;
3. Euclidean norm (or minimum distance between two points) between the simulated and measured ethanol curve.

The major interest for winemakers' is the fermentation duration. Predicting this information might allow them to act in advance on the must with nitrogen or nutrition addition (see Chapter 2). For this reason, we decided to give more importance to the first term. In fact, an experiment is said "well predicted" if one of the following statements is true:

- $| t_f^{sim} - t_f^{meas} | \leq 1.5\,\mathrm{d}$;
- $1.5\,\mathrm{d} < | t_f^{sim} - t_f^{meas} | < 2.5\,\mathrm{d} \quad \wedge \quad | E_{sim}^{fin} - E_{meas}^{fin} | \leq 4\,\mathrm{g\,L^{-1}} \quad \wedge \quad ||e||_2 < e_{mean}$.

where, $t_f^{sim}$ and $t_f^{meas}$ are the simulation and the measured curve fermentation times, respectively; $E_{sim}^{fin}$ and $E_{meas}^{fin}$ are the ethanol concentration final values of the simulation and the measured curve, respectively; $||e||_2 = \sqrt{\sum_{i=1}^{N} e_i^2}$ is the Euclidean norm between the simulated and the measured ethanol curve, where $e = E_{sim} - E_{meas}$ and N is the number of data; $e_{mean}$ is the mean value of the Euclidean norms of all the experiments.

We have decided that all the simulations which can predict the fermentation time in a range of $\pm 1.5$ d are "good", without considering the final ethanol and the error between the curves. Then, with poorer $t_f$ prediction ($\pm 2.5$ d), two other conditions are considered. The final ethanol prediction must be correct within an uncertainty of $4\,\mathrm{g\,L^{-1}}$(corresponding to 0.5% of alcohol content approximately). In addition, the Euclidean norm should be lower than its mean value among all the experiments.

Using this criteria, we have defined which experiments were "well predicted" and which were not. The model validation results are here listed:

- Considering all the experiments (except for outliers) the portion of "good predictions" is 72%.
- Considering the experiments in which $R \in [0.5 - 0.8]$ the percentage increases to 90%.
- The model found difficult to predict experiments with very low $R$ velues. In fact, for $R < 0.5$ only, 41.2% of the experiments are "well predicted".

Note that, according to [17], the initial conditions of the must are reasonably acceptable within ranges of 230-280 $\mathrm{g\,L^{-1}}$ and 150-180 $\mathrm{mg\,L^{-1}}$ for sugar and yeast assimilable nitrogen, respectively. These values correspond to $R \in [0.5 - 0.8]$. In view of this, we can conclude that the model is validated for typical winery conditions.

At this point, it is clear that the alcoholic fermentation is a complex process which comprehends a vast multitude of variables and behaviors. The reasons why certain experiments have not been "well predicted" cannot be due only to the initial value of $S_0$ and $\mathrm{YAN}_0$. Consider for example the two experiments, Dor3 and Sar4: they have very similar initial conditions values but different kinetics. However, the model cannot accurately predict the kinetic of Dor3, but it does with Sar4 (Figure 5.6). This might depend on several factors: our hypotesis is that Sar4 has a higher values of *Glutamine*; in fact, when high levels of this amino acid concentration are reached, the YAN consumption rate consider-

ably increase (according to *Ever - Italiana Biotecnologie*). Further studies comprehending amino acids and vitamins analyses will improve the accuracy of the model.

| Supplier *name* | $S_0$ *g/L* | $YAN_0$ *mg/L* | $R$ $\cdot 10^3$ |
|---|---|---|---|
| Sar4 | 272,3 | 210 | 0.77 |
| Dor3 | 278,4 | 210 | 0,75 |

Table 5.5: Values of $S_0$, $YAN_0$ and $R$ for the two experiments Sar4 and Dor3.

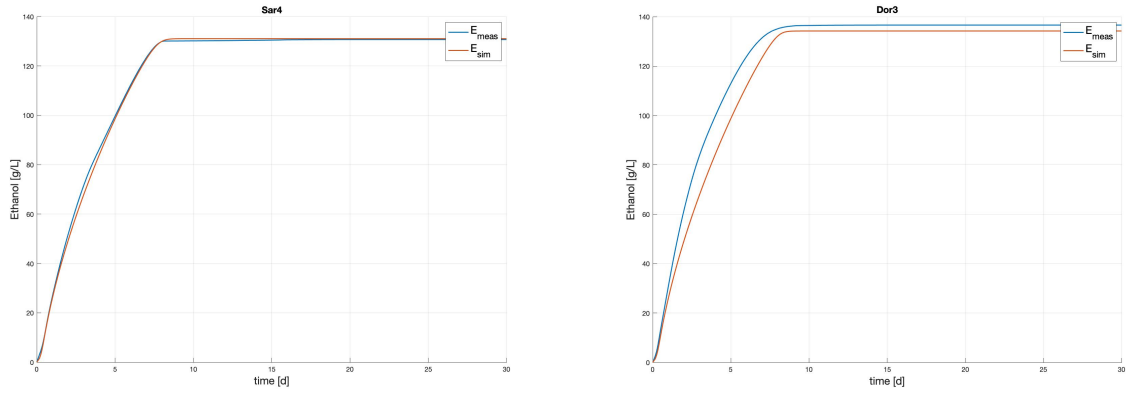


Figure 5.6: Comparison between the different kinetics and predictions for Sar4 and Dor3, having very similar initial conditions (see Table 5.5).

The result of this identification are quite satisfactory; in fact, the model can predict fermentation kinetics with good accuracy in a wide initial conditions. The predictive performance decreases with low value of $R$. For this reason sluggish fermentations are not well predicted. Nevertheless, this model identification is a promising starting point for the *Precision Winemaking Model* project.

### 5.3.2. Nanovinification 5 and Microvinification

Five nanovinification and one microvinification experiments were performed using the industrial must sample.

A significant imbalance between the industrial must sugar and YAN concentration values can be observed from the fermentation kinetics data (see Section 3.8). This imbalance

might be due to the fact that the sampling has occurred when the must was not homogeneous; our hypothesis is that various stratifications were present in the fermentation tank and the sample could not have been representative of the total mass.

The sugar initial condition ($330\,\mathrm{g\,L^{-1}}$) of the industrial must is certainly anomalous compared to both the previous experiments and the real winemaking one. As a matter of fact, the sugar mean value of the fourth period (sampled on the $15^{th}$ of December) and the one of the *Sartori* winery (sampled on the $17^{th}$ of January) are $270\,\mathrm{g\,L^{-1}}$ and $300\,\mathrm{g\,L^{-1}}$, respectively. Moreover, the YAN value of $104\,\mathrm{mg\,L^{-1}}$ is too low compared to the mean value ($185\,\mathrm{mg\,L^{-1}}$) of the fourth period experiments.

It is clear that the model validated on the nanovinification (1-4) experiments could not be applied to a must with such extreme initial conditions. For this reason, we decided to run a different identification strategy aiming to lay the foundations for a study concerning stuck and sluggish fermentation prediction. This identification strategy will be used in the optimization study presented in Section 6.

For the reasons explained above, the $k_d$ parameter was added to the identification, as mentioned in Section 5.1. The initial parameter values were the ones obtained in the previous estimation, except for $k_d$: its initial value was taken from [19]. The new estimates are listed in Table 5.6.

| Parameter | Initial Value | Estimate |
|---|---|---|
| $\mu_1$ | 2.1524 | 0.5011 |
| $\beta_1$ | 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 5.6: Parameters estimates.

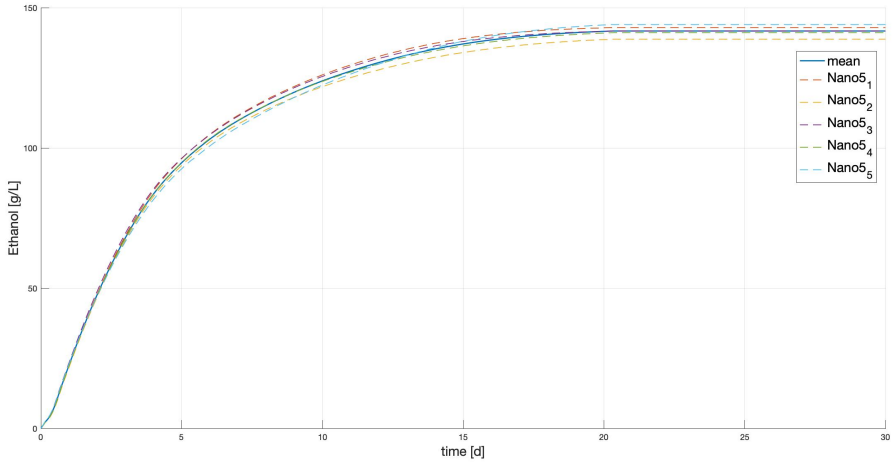


Figure 5.7: Comparison between the five nanovinification experiments and the mean curve.

The parameters were identified considering the mean curve obtained from the five nanovinification experiments. It was supposed that there was no loss of generality in doing so, since the mean error between these five kinetics and their average curve is not significant ($e_{max} = 2.28\,\mathrm{g\,L^{-1}}$), as it can be observed in Figure 5.7. Moreover, we decided to perform a single identification procedure since the nanovinification results were comparable to those obtained in microvinification, as explained in Section 3.8.

Considering this identification strategy, the model can predict the experiments kinetics (nanovinification 5 and microvinification) with satisfactory results, as depicted in Figure 5.8. However, since the model is based only on six experiments (employing the same must), it is not sufficiently validated to manage stuck and sluggish fermentations in general. Nevertheless, it was considered adequate for our optimization study, whose experiment has been carried out with the same must.

As expected, most of the parameters experienced a variation of one order of magnitude (see Table 5.6) with respect to the nominal literature values. This is totally reasonable: in fact, a single set of parameters is unlikely to be adequate to properly describe, at the same time, normal, stuck, and sluggish fermentations. Further studies, with further fermentation data, will improve the model identification.

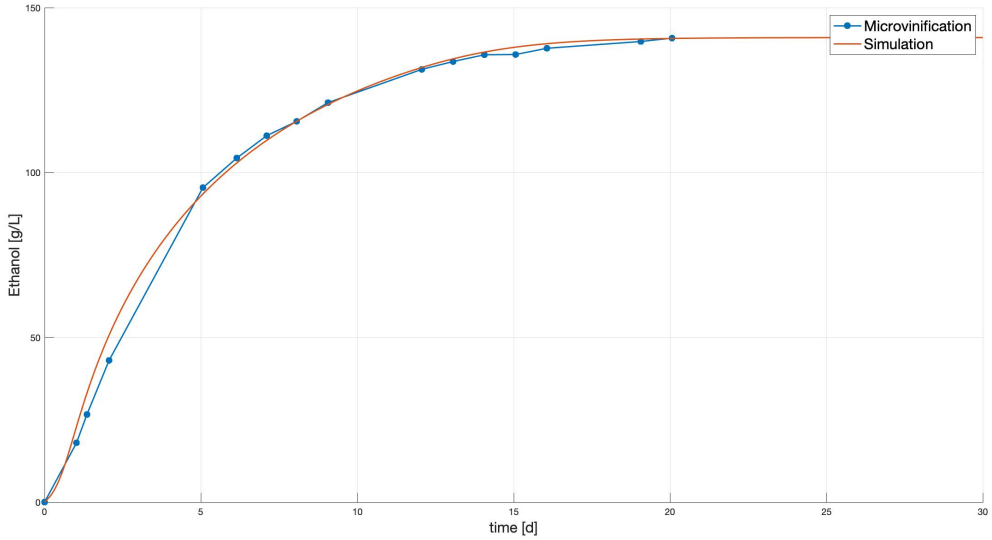


Figure 5.8: Comparison between the microvinification experiments and the model simulation.

# 6 | Optimization Strategy and Experimental Results

## 6.1. Introduction

With reference to the theme of sustainability, an important goal of the project (see Chapter 1), we decided to study the possible energy savings in relation to the fermentation process. The Schenk & Schulz model (Section 4.4.7) was considered since it had already been used in our previous studies and because it was already validated in this specific field.

The biochemical transformation of sugars into alcohol performed by the yeast is an exothermic reaction. 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. However, our work did not study the correlation between wine quality and fermentation temperature profile variations, which will be subject of future investigations.

The microvinification in jars using the industrial must sample was employed. As already mentioned in Section 3.5.2, the experiment was performed in duplicate, and the *Inverno 1936* yeast was used.

The optimization problem has been entirely implemented in the MATLAB environment. Regarding the fitting procedure (Section 6.2.2), the curve fitting app was used. Then, in order to perform the nonlinear constrained optimization, the function *fmincon* was utilized.

## 6.2. Optimization Procedure

### 6.2.1. Optimization Problem

The optimization of the fermentation process has the following threefold objectives:

- Minimization of the difference between the simulated and the desired ethanol curve.
- Minimization of the fermentation time.
- Minimization of the 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 optimization problem reads as follows:

$$\begin{aligned} \min_{x,\vec{T_u}} \quad & \gamma_1 \sum_{t=0}^{\mathcal{T}} (E_{ref}(t,t_f) - E_{sim}(t,\vec{T_u}))^2 + \alpha\ t_f + \gamma_2 \sum_{i=1}^{N} (T_{ext} - T_u(i))^2 \\ \text{s.t.} \quad & \text{Schenk \& Schulz model (4.23)}, \\ & \text{constraints (6.2a) (6.2b)}. \end{aligned} \tag{6.1}$$

In (6.1) $E_{sim}(t,\vec{T_u})$ is the simulated ethanol profile, $E_{ref}(t,t_f)$ is the desired ethanol behaviour, $t_f$ 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), $\mathcal{T}$ represents the fixed simulation time horizon of 20 *days* and $N$ is the number of sampling steps in which it was decided to divide the time horizon. Furthermore, $\gamma_1$, $\gamma_2$ and $\alpha$ are suitable weights. In addition, $p$ is the set of parameters estimated from the previous nanovinification experiments (see Section 5.3). Therefore, in formulation (6.1), the first term represents the tracking error, the second one the fermentation time, and the third one the energy consumption. Constraints have been added regarding the maximum and minimum temperature values, and its variations, see Table 6.1. These values were suggested by the *Ever - Italiana Biotecnologie* staff. The constraints have been introduced as follows:

$$T_{min} \leq T_u(i) \leq T_{max} \qquad \forall i = 1, ..., N \tag{6.2a}$$

$$\Delta T_{min} \leq T_u(i) - T_u(i-1) \leq \Delta T_{max} \qquad \forall i = 2, ..., N \tag{6.2b}$$

| $T_{min}$ | $T_{max}$ | $\Delta T_{min}$ | $\Delta T_{max}$ |
|---|---|---|---|
| 15 °C | 28 °C | −2 °C | 2 °C |

Table 6.1: Values for the constraints in the optimization problem.

### 6.2.2. Strategic Choices

#### Temperature Profile and Sampling Time

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. This means that, for each sampling step $k$, a degree of freedom is added, that is, a decision-making variable, $\vec{T_u}(k)$, to be kept constant for a period denoted $\tau_k$, as in Figure 6.1.

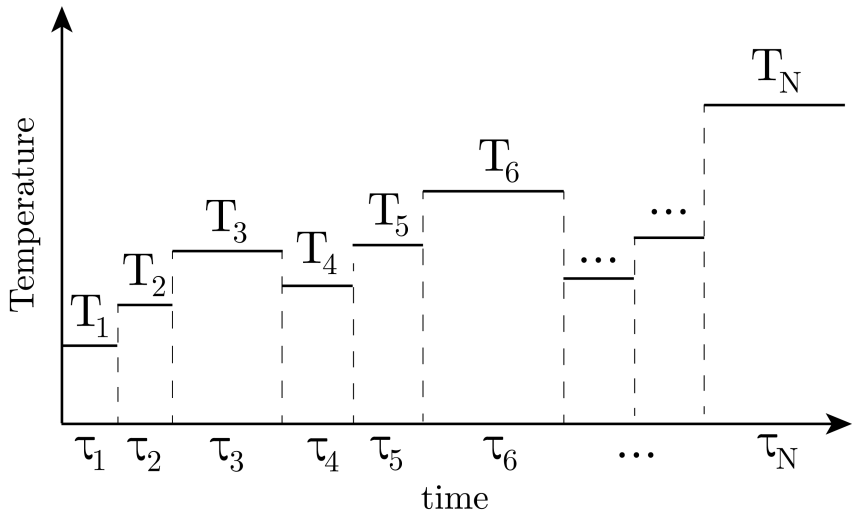


Figure 6.1: Example of free variables in the temperature profile.

A variable sampling time has been selected. More specifically $\tau_k$ 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 chosen sampling instants are described in Table 6.2. Namely, in the first week, the sampling instants correspond to 8:00, 13:00, and 18:00 each day. On the other hand, in the second week, control inputs could be varied just two times a day (i.e., 8:00 and 18:00).

| Week | Time | | |
|---|---|---|---|
| 1 | 08:00 | 13:00 | 18:00 |
| 2 | 08:00 | - | 18:00 |

Table 6.2: Chosen sampling time.

The choice of the sampling times has been done, not only based on physical considerations, but also for practical ones (i.e., the temperature values had to be changed manually by *Ever - Italiana Biotecnologie* staff) and numerical considerations (minimization of the computational complexity).

## Desired Ethanol Curve and End Fermentation Time

Once the sampling time and the type of the input profile are defined, we now specify the optimization goals in details.

In particular, denote with $\vec{z}$ the vector whose elements are the components of the cost function defined in (6.1), i.e.,

$$\vec{z} = \begin{bmatrix} E_{ref} - E_{sim} \\ t_f \\ T_{ext} - \vec{T_u} \end{bmatrix} \tag{6.3}$$

Instead, in this work, 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 $t_f$. For fitting purposes, the ethanol curve obtained from the nanovinification experiments was used for defining the shape of the reference one. This curve was fitted using the following sigmoidal function:

$$f(t) = \frac{t^b}{t^b + c^b} a \tag{6.4}$$

where $a = 149.9$, $b = 1.644$ and $c = 3.646$.

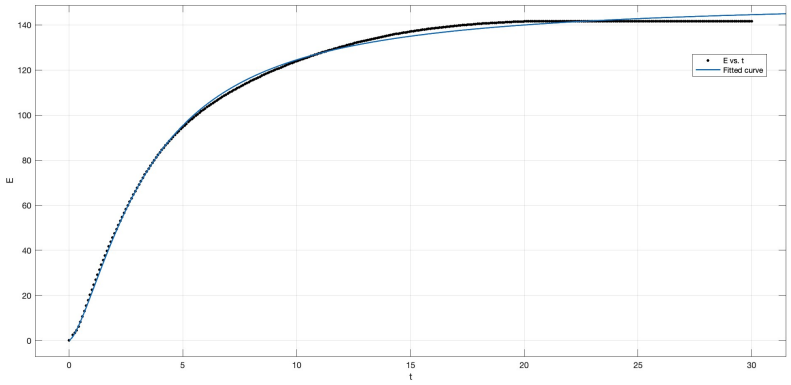


Figure 6.2: Ideal ethanol curve vs fitted curve (6.4).

Figure 6.2 shows the comparison of the two curves. In nanovinification experiments, the fermentation time was approximately 20 *days* (the computation of this term is described in Section 3.6). Therefore, the variable $t$ inside function (6.4) has been replaced with the term $\frac{20}{t_f}t$. This change of variable in the curve allows the optimization algorithm to change as a function of $t_f$. In Figure 6.3 we show how the fitted curve changes considering different values of $t_f$. The desired ethanol curve, then, has been defined as:

$$E_{ref}(t, t_f) = \frac{(\frac{20}{t_f}t)^b}{(\frac{20}{t_f}t)^b + c^b}a \tag{6.5}$$

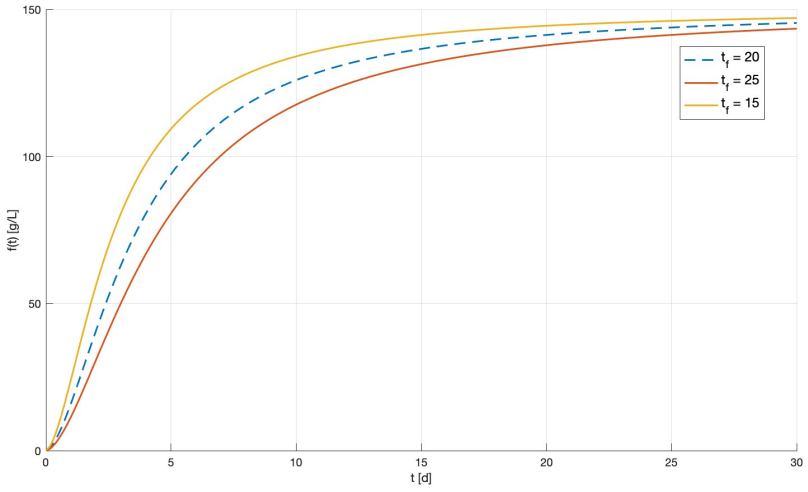


Figure 6.3: Fitted curve with different fermentation times.

## Energy Consumption

The energy consumption is mainly due to the necessity of cooling the tank to contain the tumultuous phase of the fermentation. Therefore, we decided to set a maximum temperature of the fermentation mass at 28 °C before cooling. We assumed that the external temperature was constant at 22 °C and that there was a direct proportionality between the energy consumption and the difference in the external and internal temperature.

## Weights

The cost function needs to be formulated in such a way that the simulated curve follows the fitted curve. Note that, if there was no constraint on $T_u(k)$ and a cost on the used energy, the algorithm would try to reduce $t_f$. A weight was assigned to each of the three terms that form the cost function (6.1), and the best trade-off was found via a trial-and-error procedure. The chosen weights are listed in Table 6.3.

| Weight | Value |
|---|---|
| $\gamma_1$ | 10 |
| $\gamma_2$ | 5 |
| $\alpha$ | $10^6$ |

Table 6.3: Weights.

In the paper [19], several oenological indications were given, i.e., to set $t_f = 20\,\mathrm{d}$ and to consume the sugar as linearly as possible up to the final value of $18\,\mathrm{g\,L^{-1}}$.

As can be noted from the weight values (Table 6.3), we decided to gave a higher priority the tracking and the fermentation time goals for the following reasons:

1. The working routine of *Ever - Italiana Biotecnologie* has forced us to limit the time of the test to 15 days, against the 20 days of the previous experiment.
2. The uncertainty about the temperature of the room where the experiment was carried out was extremely high. In fact, since it fluctuated around 18 °C at night and 22 °C during the day, it was not possible to measure it accurately. Given this problem, an accurate estimation of the energy savings was not possible. In view of this, we decided to partially discard it from our cost function.

## 6.3. Results

The temperature profile $\vec{T_u}$, obtained as the result of the optimization problem, is shown in Table 6.4 and in Figure 6.4. Note that, in Table 6.4, only the sample instants at which $T_u(k)$ value varies are reported. The comparison between the simulated ethanol curve (using $\vec{T_u}$) and the desired one is shown in Figure 6.5, where the optimal fermentation time is $t_f = 15.62\,\text{d}$. Note that, thanks to the suitable values chosen for $\gamma_1$ and $\alpha$, the simulated curve follows the desired curve quite accurately.

| Date | Time | T |
|---|---|---|
| 28/02 | 18:00 | 21 °C |
| 01/03 | 13:00 | 23 °C |
| 01/03 | 18:00 | 25 °C |
| 02/03 | 08:00 | 28 °C |
| 07/03 | 08:00 | 27 °C |
| 10/03 | 13:00 | 24 °C |
| 11/03 | 13:00 | 22 °C |

Table 6.4: Optimal temperature profile.

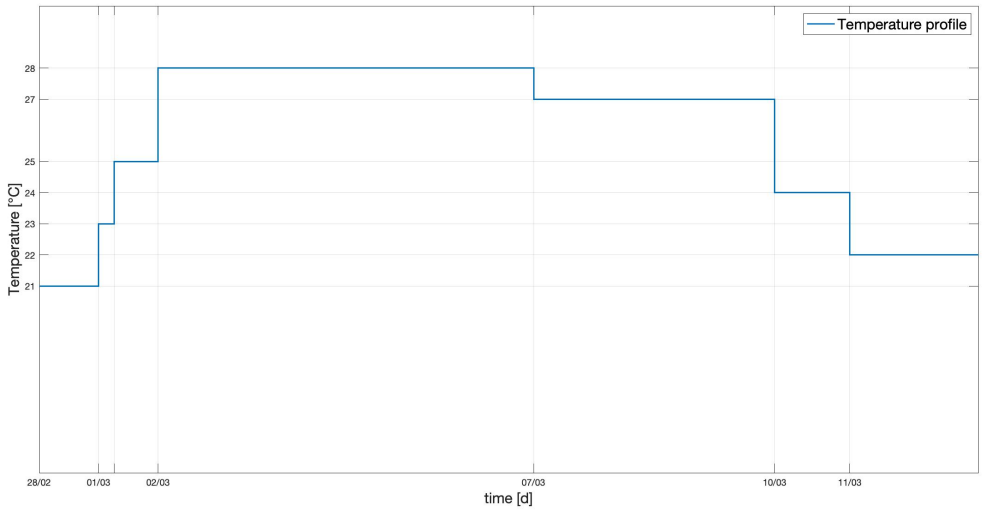


Figure 6.4: Optimal temperature profile graph.

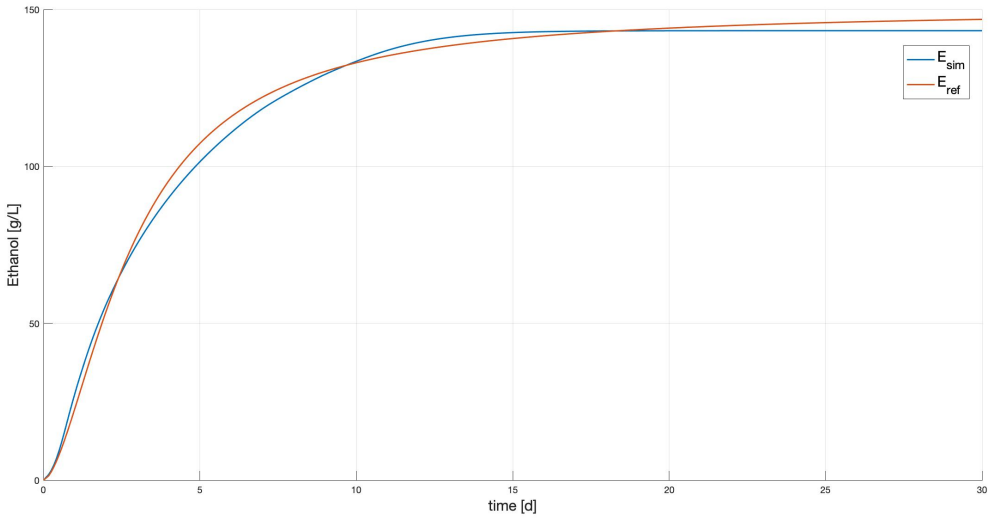


Figure 6.5: Comparison between the desired ethanol curve $E_{ref}(t, t_f)$ and the simulated ethanol curve $E_{sim}(t, \vec{T_u})$.

The comparison between the simulation and the microvinification experiment is shown in Figure 6.6. The experimental ethanol curve behaves consistently with the simulated one in the yeast growth phase until about the third day. However, from this moment on, the fermentation speed begins to decrease, and the consistency with respect to the simulation is lost. 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, in reference to the behavior of the function $\mu(N, T)$ described by David & Dochain (Section 4.4.6), when $YAN_0$ is low, the yeast grows rapidly consuming almost all the nitrogen; high temperatures tend to intensify this behavior. This is confirmed by our experimental results, as can be observed in Figure 6.7.

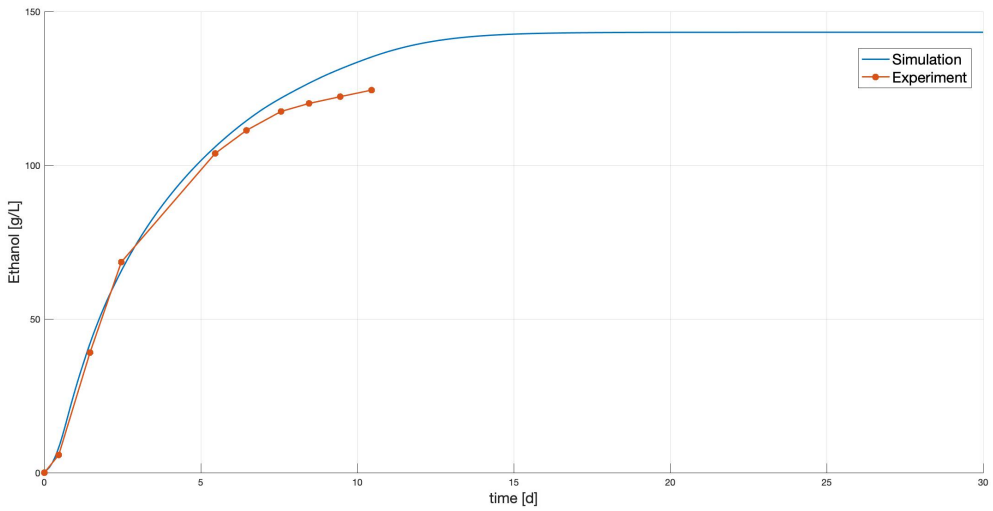


Figure 6.6: Comparison between simulation with the optimal $\vec{T_u}$ and microvinification experiment.

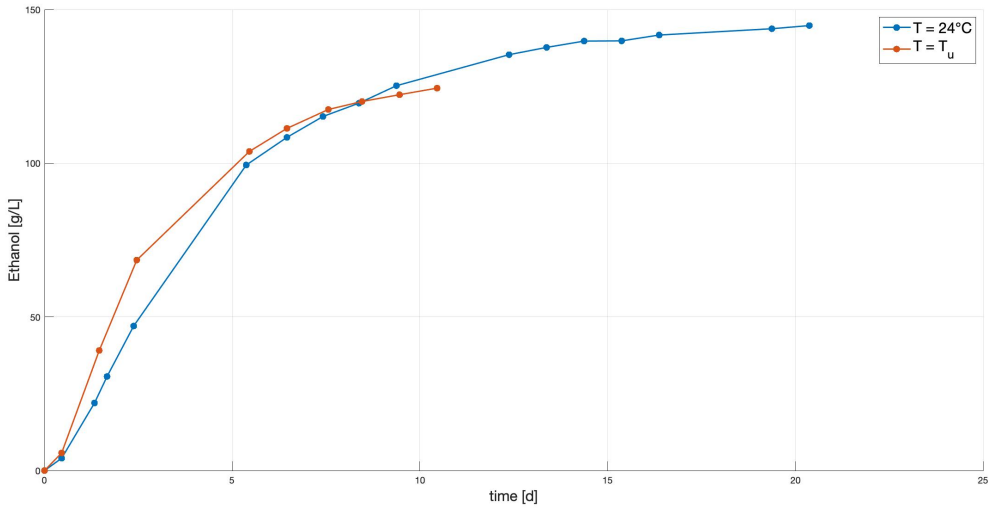


Figure 6.7: Comparison between the experiments in jar, one with the fixed temperature (24 °C), one with $\vec{T_u}$ applied.

The goal of the optimization study was to investigate the behavior of fermentative kinetics and to validate our identification of the mathematical model under conditions of variable temperature 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. Therefore, the outcome of this study can be a basis for improving the identification of the model parameters in the future.

Also, due to the current inadequacy of the available instrumentation, we could not apply closed-loop strategies (i.e., MPC) to control the fermentation dynamics. This will be subject of future works.

# 7 | Conclusions

This thesis is a case study that lays the foundations for the implementation of a predictive model for the alcoholic fermentation of the *Amarone della Valpolicella DOCG* wine. The project has been devised in the framework of the technological cluster *ALL4INNOVATION*.

The thesis has been functional in creating a methodological base to help wine companies in the *technological transformation* aiming to improve the quality of wine and obtain a lower energy consumption. 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 is still not suitable to predict the fermentation performances of the *Amarone* wine in view of the following reasons: the winery conditions are not well represented by the model terms (absence of pomace, fermentation temperature gradient, oxygenation, etc.), the sugar, yeast, YAN profiles are not measured, absence of terms representing the addition of nitrogen during fermentation. However, this thesis has laid robust 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, optimizing human, physical, economical resources without losing in reliability. The nanovinification process seemed the most promising solution in terms of data numerosity, cost savings and performance. The performance indices of the fermentation kinetics were consistent with the research and technology partners' (*Ever - Italiana Biotecnologie*) experience. However, the downside of the nanovinification process is the absence of grape solids in the fermentation bottle during fermentations. To overcome the small-scale limitations (Section 3.7), experiments including pomace and sensors (online and offline) will be conducted in the future. In this

perspective, the collaboration with *Università di Verona - Viticoltura ed Enologia - Dipartimento di Biotecnologie* is very promising. They could perform laboratory vinification experiments in 3L quantity with inclusion of pomace. The comparison (analytical and sensorial) of this procedure with the industrial one gives excellent results.

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** The experimental data robustness is significantly determined by this phase. In this perspective, the project needs a more accurate planning in terms of pallet lay out, samples' storage, sampling methods, etc.

**YAN Analysis Methods** The YAN measurement difference between FOSS and UPLC analysis methods is presented in Section 3.2.3. In particular, the FOSS method tends to overestimate measurements. In the future, a study will be necessary to confirm the UPLC as the reference measurement method for YAN analysis. The alignment of the measurement obtained with the three most common YAN analysis methods (FOSS, Enzymatic method, UPLC) will allow the wineries to choose the most agile and cost-efficient methods for this kind of analysis.

**Instrumentation** Including sensors to the fermenters will allow an online data collection for fermentation variables (e.g. temperature, oxygen, density, carbon dioxide etc.). This will also be useful in view of control action implementation. Moreover, sampling and multiparametric analysis systems will be implemented for the offline measure of sugars, yeast assimilable nitrogen and microbiological yeast measurement during fermentation.

**Mathematical Modelling** A more thorough and detailed study of fermentative process modelling must be pursued in order to make it an accurate and reliable tool. In this perspective, the future work will consist of the following points:

- To properly model the red wine fermentation, a heat and mass transfer study in heterogeneous wine fermenters and an investigation on phenolic extraction during

fermentation must be addressed. Quantitative modelling of these concepts allows for improved physical understanding and process optimization.

- The yeast cells' metabolism modelling must be improved; in this perspective, recent research in this field concerning "structured" and "flux-based" models, must be studied. These models try to account for the myriad of processes within the yeast cells and serve as a transition between the unstructured models (the ones presented in this thesis) and the more complex cell metabolism modelling.
- Data-based models, which use machine learning and multivariate analysis, must be investigated.
- Stuck and sluggish fermentation suitable model must be devised ad hoc.
- Choosing the best modelling option according to the different raw material and production process: vinification of white, red, rose, sweet, sparkling wine, and others.

**Technological Support** A suitable data storage and elaboration system should be implemented. This could provide a technological support for the sensor-data collection, and could be a valuable support for the winery process management. In this perspective, the collaboration with the technological partner *APRA* is very promising.

In conclusion, the thesis results have proven that the *Precision Winemaking Model* project may set the conditions to create a predictive models cluster aiming to manage the fermentation process. This diagnosis-management-prediction system is set to become the business transformation standard for the winery production processes.

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