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Modeling and Supervision of the Beer Fermentation Process

Control Lab of the Engineering Faculty of UMONS

Master Thesis
Presented with a view to obtain the grade of Master's in Electrical Engineering: Specialist Focus on Signals, Systems and BioEngineering

Amanda BESEN BORGES

UNIVERSIDADE FEDERAL DE SANTA CATARINA
Centro Tecnológico - CTC

POLYTECH MONS

Academic Promoters:
A. VANDE WOUWER
L. DEWASME
D. COUTINHO (UFSC)
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Acronyms

Sign	Description	Page
OE	Original Extract	4, 6, 29
RE	Real Extract	4, 6
$A_{w/w}$	Alcohol	4
ODE	Ordinary Differential Equations	5, 6, 8, 17, 67
CTR	Carbon Dioxide Transfer Rate	18, 29, 31, 33, 37, 46, 47
EKF	Extended Kalman Filter	26, 27, 31, 46
P	Predicted Covariance Matrix	27, 28
IDE	Integrated Development Environment	33, 82, 83
ABV	Alcohol by Volume	79
SI	International System of Units	79
UUID	Universally Unique Identifier	82

Abstract

Water, malt, hops and yeast are the main ingredients to do beer, but that is not all that good beer is made off. A good beer is made off a detailed recipe with a strongly controlled production assisted by several quality control systems.
To make beer, a Brewer must pass through some processes, fermentation being one of the most important of them. This process is responsible for transforming wort into beer, and several variables can affect it significantly, such as temperature, the age of yeast strains and ethanol concentration. Hence, it is important to control the process.
This master thesis final work has the objective of designing a monitoring system for a fermentation process in beer brewing. Firstly, several models available in the specialised literature of batch beer fermentation process are reviewed. Then, an original model is proposed in order to take the available on-line measurements into account. Modelling is based on the stoichiometric equation for the yeast fermentation process and it describes the production of ethanol and carbon dioxide from the consumption of substrate.
To monitor the system an observability analysis is initially carried out considering the available measurements. Then, an extended Kalman filter is designed to estimate the ethanol and biomass concentrations assuming that the substrate concentration and carbon dioxide transfer rate are available on-line through adequate sensors. A discussion about practical observability and local observability along the trajectory is carried at the end.
Experimental studies are also developed in the framework of this project, where two acquisition devices were developed, one for specific gravity and one for the emitted carbon dioxide rate, respectively. In addition, a modified sensor based on counting the emitted bubbles of carbon dioxide has been devised in order to measure the outlet carbon dioxide rate. Unfortunately, the acquired data is not conclusive and more experiments need to be achieved in the future.

Keywords: Alcoholic fermentation modelling; Sensor; Extended Kalman Filter; Bioprocess;

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Chapter 1

Introduction

The origins of beer production are not precise, but some archaeological evidences of residues of beer date from 8500 BC during the pre-pottery neolithic in Turkey. Beer production is also reported in old Egypt, Iran, Israel and Iraq. Beer in that time was not what beer is nowadays, it was a fermented liquid out of grains with no bubbles, and this recipe did not change for a long time. The first food-quality regulation law was in beer production, and it came in 1516 by the Duke of Bavaria imposing the purity law in beer, that allowed only water, hops and malted barley as ingredients of beer. With the industrial revolution the craft breweries moved to industrial manufacturers and at the end of the 19^{th} century the development of hydrometers and thermometers revolutionized the brewing industry with more accuracy in the processes. Also in the same time, another revolution occurred, the use of strain of yeast for the first time by Gamle Carlsberg. The quality of the production associated with the evolution of the process and selection of the ingredients made the modern beer. This reflected in the public appreciation and consumption of it. Ever since then, the brewing industry has been growing. Beer is one of the world's oldest prepared drinks and the most widely consumed alcohol drink in the world.

This work is dedicated to the development of monitoring systems in fermentation process of beer brewing. In the following chapter, it is presented a motivation for this study as well as an introduction of beer production, a summary of the state of the arts and some analysis of representative dynamic models for this system.

1.1 Motivation

The fermentation process is a bioreaction in batch mode having a complex set of enzymatic reactions in an exothermic behaviour that many variables can affect. This process is responsible to transform the sugars in the wort in alcohol and gas, and an error in this process is critical to the production. So measuring these variables is essential.
Most of these variables, nowadays, are measured off-line and require beer samples for analysis. It is common to extract part of the fermentation liquid to measure specific gravity. A current problem for this measurement is that the bioreactors, called fermenters, can't be opened during the fermentation process because the inoculated wort cannot have contact with air due to the presence of oxygen and micro-organisms such as bacteria and wild-yeast. This air can stop the fermentation or contaminate the production.
The measurements not only enable the Brewer to identify problems in the production, like contamination and yeast behaviour, but also to predict the sugar consumption and the beer characteristics like alcohol percentage and flavour characteristics.
The possibility of monitoring the fermentation process without evasive methods of subtracting measurements is interesting. With a set of selected non-evasive measurements, like the temperature or the emitted CO_2, it might be possible to observe the rest of the variables, like the sugar

concentration or the ethanol percentage, and monitor the beer so the Brewer would have a real-time monitoring system as an auxiliary production tool.

1.2 Beer Production

Beer is basically made with four ingredients: water, malt, hops and yeast. Without one of them, one cannot make beer, and every single one of them gives important characteristics to the beer. These aspects are detailed in Appendix A.
Certain knowledge, like recipes or specific strains of yeasts are hidden secrets of brewers passed through generations of family breweries but the basics are well documented. Books like *How to Brew* of J. J. Palmer [1] are dedicated to explain and guide inquisitive ones on how to brew a proper beer.
To brew a beer, one must pass through four main processes: the malting, wort production, fermentation and downstream processing. The malting process is the only one that most of the brewers don't do. This process is achieved by the malt producers.

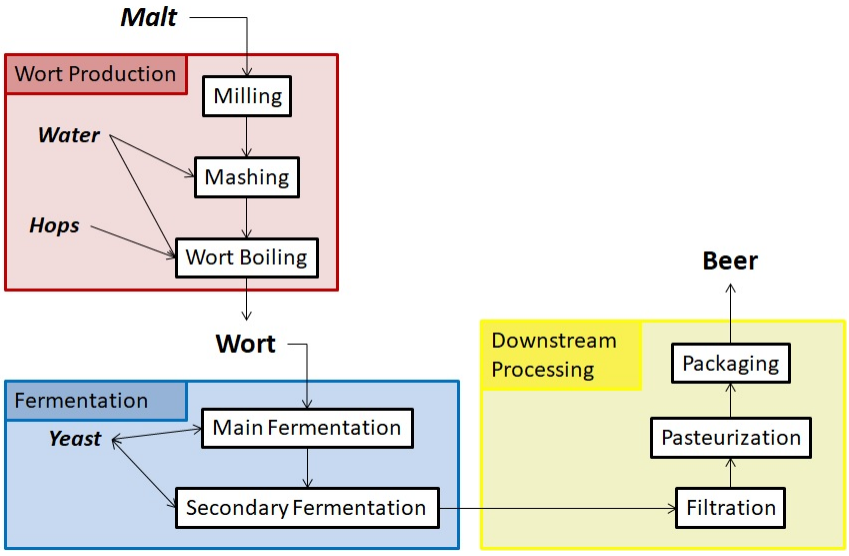


Figure 1.1: Brewing process schematics

To Start the process of brewing one must have a brewing kit, the ingredients and the recipe. Following Figure 1.1 the first step is to mill the malt and the grains. For more details the reader is referred to Appendix A. When all is milled properly, it is time to add the water and the cooking starts.
The mashing is when one rises temperature of the mashing water little by little in determined step times. Temperature and pH are controled during this process and after it finishes it is time to drain the liquid to start the boiling.
Liquid separated from the grains will continue the process and will be boiled for about one hour. At the boiling, it is added the hops and some herbs, spices or fruits depending on the recipe. At the end of the boiling process, the Brewer will have its wort at a temperature that is inappropriate for the yeast survival. So the next step is to cool it down.
One must cool the wort down very fast, to avoid contamination. When the wort reaches the ideal temperature for the yeast, the Brewer will add the liquid to the fermenter to start the fermentation process. After adding the yeast to the fermenter and locking it properly the main fermentation can start.
The main fermentation takes normally seven to ten days for the most common beers and after that the Brewer can choose to do two things: add more sugar to start a second fermentation in

the bottle (priming process); or continue the process in the fermenter.
If one chooses to continue the process, the liquid passes by a secondary fermentation, or a maturation, where the Brewer can add more hops, spices and fruits. This secondary fermentation can take different times, depending basically on the style of the beer and the recipe of the Brewer. Filtering can be achieved right after to extract the big particles like the extra ingredients and the biomass.
A big debate in the brewing industry is the need of the Pasteurization process, which is done by heating the beer to inhibit the growth of micro-organisms inside the beer like yeast and bacteria, to prevent spoilage, over fermentation and contamination. Most industrial beers are pasteurized, while homebrewed beers are not.
To end the brewing process, its time to pack the beer, which can be done by bottling, kegging or plumbing directly to the consumer. An important observation is if one chose to do the secondary fermentation inside the keg or bottle, than one must wait at least two weeks to finish the secondary fermentation before drinking the beer, called priming process, to have a nice foam and bubbles in the cup instead of a sweet juice beer flavour.

1.2.1 Fermentation

The process studied in this project is the main fermentation. Fermentation is a biochemical process that extracts energy from a carbohydrate through the action of enzymes in the absence of respiration [1]. The main purpose of the chemical Reaction 1.1 is to get energy, but its waste is the one brewers are looking for, alcohol and CO_2. Also, following [2], the starting sugar may not be glucose, but the reactions are similar or the sugar will break into glucose at the end.

$$C_6H_{12}O_6 \xrightarrow{yeast} 2C_2H_5OH + 2CO_2 + energy \tag{1.1}$$

During the first hours of the fermentation process, in theory, it is possible for the Reaction 1.2 (aerobic fermentation) to occur because of the soluble oxygen in the wort. Following [3], the Saccharomyces cerevisiae in sugar concentrations over 1.040 will do the anaerobic respiration (Reaction 1.1) even in the presence of oxygen, this phenomena is called the Crabtree effect.

$$C_6H_{12}O_6 + 6O_2 \xrightarrow{yeast} 6CO_2 + 6H_2O + energy \tag{1.2}$$

Due to the catabolite repression, explained in [2], the cells will be repressed and won't engage in aerobic respiration. The main use of the oxygen, in this particular case, is to grow cell membranes for enlarging the number of cells. Even if there is aerobic respiration in parallel of the Crabtree effect, it is an irrelevant reaction. The main reason why is that the oxygen consumption is extremely fast compared to the total quantity of carbon dioxide emitted.
For this reason, most papers that elaborate on fermentation of yeast will use Equation 1.1 as their main stoichiometric equation. Based on this equations kinetic models can be proposed. Some models also add energy balances to compensate the temperature influence.

1.3 State of the art

For control engineers, system modelling is the derivative of mathematical models to simulate a real system [4]. In this section, one will summarize some studies in the scientific research on beer, especially on mathematical modelling.

1.3.1 Balling Formula

In the 1800s, the chemist Karl Balling developed a static model to describe the relations among beer brewing components [5]. In his work, a series of tables were presented describing the relationship between Original Extract (OE), Real Extract (RE) and Alcohol ($A_{w/w}$). Based on these results, two relationships can be established:

- The first says that for every 1.0665g of extract 1g of alcohol, 0.9565g of carbon dioxyde and 0.11g of biomass can be obtained:

$$\underset{extract}{2.0665g} \rightarrow \underset{alcohol}{1.000g} + \underset{CO_2}{0.9565g} + \underset{biomass}{0.11g} \tag{1.3}$$

- The second is a derivation of the first one:

$$OE = \frac{100\ (2.0665\ A_{w/w} + RE)}{100 + 1.0665\ A_{w/w}} \tag{1.4}$$

The relationship between Original extract, real extract, apparent extract and alcohol in beer production was studied not only by Balling but also by Wahl and Henius [6] in 1908, Holtzer [7] in 1904 and others. These works are controversial because of their assumptions and approximations. A critical study is [8] by A. J. Cutaia, A-J Reid and E. A. Speers, where it is possible to find the relationship between these brewing values and their errors.
Even though, these works are in widespread use in the industry and give a great start for the development of new technologies. For example, in order to predict the quantity produced of CO_2, its yields, the amount of carbohydrates used in the process and other parameters I. S. Daoub and B. A. Searle, in [9], use the Balling equations. They discuss the monitoring of a fermentation process by measuring the evolution of CO_2. In their paper there is the correlation between the CO_2 rate with respect to other fermentation parameters like Yeast concentration, specific gravity and ethanol. They also analyse the response of CO_2 to different fermentation conditions and develop an on-line metering system for evolved CO_2 rate in bioreactors.
Another great example is in [10] where once again the yields and two other parameters are calculated using the Bailling equation. In this paper, I. Parcunev *et al.* present a method to determine the kinetic parameters of the models for different fermentation conditions.

1.3.2 Kinetic Models

The kinetics modelling is one of the most common types of biochemical reaction modelling. It is usually associated with mass balances and presents the evolution of chemical compounds over time. In the brewery industry it is commonly used for modelling the behaviour over time of the component concentrations of the stoichiometric equations such as Equation 1.1.
A good example of the kinetics models used for brewery fermentation is the work of W. F. Ramirez and D. A. Gee. In 1987 they presented in [11] their mathematical model based on a previous work of Engasser et al. [12] where they include the temperature effects. They used optimal control theory to optimize the performance of the ethanol production and reduce batch time. The optimal control law is a bang-bang control with respect to temperature.
In 1994 D. A. Gee and W. F. Ramirez enlarged their model adding flavour aspects. In [13] they added aminoacids compounds, fusel alcohols, esters, vicinal diketones and acetaldehyde and discussed the results of the parameter identification of the new system.

In 1996 they simulated, tested and used an extended Kalman filter and a recursive prediction error method to estimate states variables and identify parameters in [14]. In 2007 W. F. Ramirez and J. Maciejowski introduced a new tool to determinate the optimal cooling policy to maximize the ethanol production in a constrained time and optimize flavour characteristics.
B. de Andrés-Toro *et al.* in [15] discuss a different kinetic model. Their model takes in consideration the phases of the biomass (lag, active and dead cells), sugar, ethanol, diacetyl and ethyl acetate. Also they made experimental analysis in laboratory with isothermal conditions and in industrial scale with temperature changes during time and they proved that their model has a good validity in both situations.

1.3.3 Dynamic Models

Another type of modelling the fermentation rates is by adding dynamic models based on neural networks to predict some brewing values. In [16] G. Corrieu, I. C. Trelea and B. Perret use the measurement of CO_2 to on-line estimate the fermentation rate, the wort density and the ethanol concentration and also implemented a dynamic model based on neural networks to predict the future density evolution and the fermentation end-time.
A year later, I.C. Trelea and G. Corrieu *et al.* published [17] where they presented and compared three dynamic models for beer fermentation that takes into account temperature, top pressure and initial yeast concentration and predicted density, residual sugar concentration, ethanol concentration, and released CO_2.
Another paper from I.C. Trelea, G. Corrieu *et al.* [18] presents a model of beer fermentation in terms of flavour components using temperature, pressure and yeast inoculum as factors and a CO_2 emission measurement for prediction of aroma associated with these factors.
A. D. Rodman and D. I. Gerogiorgis in [19] used dynamic simulations to analyse the effect of initial conditions and concentrations of the beer parameters like sugar and pitching rate and the effect of different temperature profiles in the flavour and beer quality.

1.4 Representative dynamic models

To deepen the analysis of the system, it was selected three representative models in the bibliographic references. They are implemented and tested for a final discussion about the technical aspects and their advantages and disadvantages.

1.4.1 Balling Based Model

The model of the article [10] was chosen for its simplicity. This paper proposes two models, the first one is a system of Ordinary Differential Equations (ODE) derived from the Balling Equation 1.4. The second one is a simplification of the first one where a system of linear functions is used to extract the information on substrate, ethanol and biomass from the CO_2 evolution. A summary of both models can be seen in Appendix A in Subsection A.3.1.
For the ODE model, firstly the yields of the chemical compounds with respect to the substrate is calculated using the Balling Equation 1.4. Also from the Equation 1.4 it's possible to derive the system of ODE that will be used for the biomass (X), ethanol (E) and extract (S).

$$\frac{dX}{dt} = \mu(t)X(t) \tag{1.5}$$

$$\frac{dE}{dt} = q(t)X(t) \tag{1.6}$$

$$\frac{dS}{dt} = -\frac{1}{Y_{\mathrm{X/S}}}\frac{dX}{dt} - \frac{1}{Y_{\mathrm{E/S}}}\frac{dE}{dt} \tag{1.7}$$

The specific growth rate μ(t) and the product accumulation rate q(t) for this model are described with three different well known kinetic mathematical models: Monod [20], Aiba [21] and Tessier [22].

Monod	**Aiba**	**Tessier**
$\mu(t) = \mu_{\max}\frac{S(t)}{K_{\mathrm{SX}}+S(t)}$	$\mu(t) = \mu_{\max}\frac{S(t)}{K_{\mathrm{SX}}+S(t)}e^{-K_{\mathrm{iX}}E}$	$\mu(t) = \mu_{\max}(1-e^{-\frac{S(t)}{K_{\mathrm{SX}}}})$
$q(t) = q_{\mathrm{Emax}}\frac{S(t)}{K_{\mathrm{SE}}+S(t)}$	$q(t) = q_{\mathrm{Emax}}\frac{S(t)}{K_{\mathrm{SE}}+S(t)}e^{-K_{\mathrm{iE}}E}$	$q(t) = q_{\mathrm{Emax}}(1-e^{-\frac{S(t)}{K_{\mathrm{SE}}}})$

Table 1.1: Specific growth rate and product accumulation rate

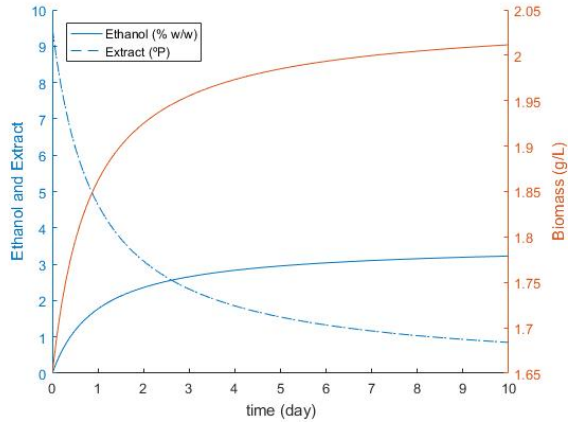
To generate the algebraic linear model, one uses the ODE from the previous model and assumes Gay-Lussac relationships. The concentrations of ethanol and substrate can be deduced from the amount of carbon dioxide. The biomass is calculated as a function of the wort ($S(t)_i$) and the OE ($S(t)_{i-1}$).

$$S(t) = S_{int} - aCO_2(t) \tag{1.8}$$

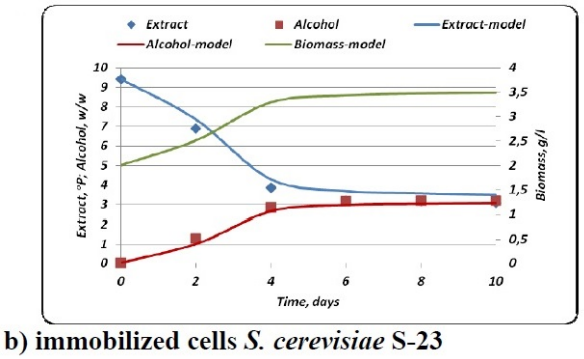
$$E(t) = b(S_{int} - S(t)) \tag{1.9}$$

$$X(t) = 0.1[S(t)_{i-1} - S(t)_i] \tag{1.10}$$

Where S_{int} is the OE, $S(t)$ is RE, b is the yield coefficient $Y_{E/S}$ and a is a coefficient that can be determined experimentally, sucha as b, but in this case it was chosen to be the same value as b.



(a)



b) immobilized cells *S. cerevisiae* S-23

(b)

Figure 1.2: Fermentation process simulated over 10 days: (a) Model Simulation; (b) Comparison between model and experimental results (eq. 4, 4a, 4b); OE=9°P. [10].

To simulate this model the ODE system is implemented in a MATLAB® script using a non-stiff differential equation solver (ode45 [23]) function and the parameters are taken all from the article [10].
To make a comparison, the figure of article [10] is reproduced, which is Figure 1.2b. Since the figure presented in the article does not give its parameters, only the name of the yeast and its original extract percentage, the values are estimated using the tables found in the same article. This model was created with the propose to analyse the parameters with respect to the different kinetic models. So, the kinetic model chosen for this discussion is Aibas, since it provides simulating results closer to the one from [10], but the other kinetics are also calculated and their comparison graphs can be seen in Appendix A in Subsection A.3.1.3.

Figure 1.2a is the result of the MATLAB® script, as it is observed, the behaviour of the components in the fermentation process are as expected. The substrate is consumed, reducing the specific gravity, while ethanol and biomass is produced in different rates.
Figure (a) and (b) have the same qualitative features in terms of initial conditions and trends of the curves (slopes and plateaus), but it is difficult to go beyond this observation as we do not know the kinetic models used to generate Sub-figure 1.2b.

1.4.2 Ramirez & Gee Model

Since the first article [11] the mathematical model suffered some small changes. For this reason, it was chosen to use all the papers of Gee and Ramirez to find all the parameters and to analyse better the model. So, to make it as accurate as possible, the article [11] was chosen as the main one and the others as complementary.
The model of Ramirez and Gee introduced in [11] has a flavour modeling but since there is no need for the flavour variables, the model that will be presented is the growth model and it has only the state variables of Glucose (G), Maltose (M), Maltotriose(N), Biomass (X), Ethanol (E) and Temperature (T).
The stoichiometric Equation 1.1 was used to calculate the mass balance of the chemical compounds while an energy balance was used to calculate the temperature flow. A summary of the model of Gee and Ramirez can be seen in Appendix A in Subsection A.3.2. A reaction scheme was elaborated, based on [24] to understand better the mass balance.

$$k_G G \xrightarrow{\mu_1 X} k_{EG} E + k_{CO_2 G} CO_2 + k_{XG} X \tag{1.11}$$

$$k_M M \xrightarrow{\mu_2 X} k_{EM} E + k_{CO_2 M} CO_2 + k_{XM} X \tag{1.12}$$

$$k_N N \xrightarrow{\mu_3 X} k_{EN} E + k_{CO_2 N} CO_2 + k_{XN} X \tag{1.13}$$

Starting with the carbohydrates, Gee and Ramirez introduced three sugars, the Glucose (G), Maltose (M) and Maltotriose (N). A good remark is that checking the Equation 1.1 it is not evident the presence of maltose neither maltotriose, this is due to the fact that maltose is a combination of two glucose and maltotriose three. They are bigger carbohydrates and the yeast will demand more energy to break them down to molecules of glucose. Because of these, the yeast will prefer to use the glucose first, than the maltose and at last the maltotriose, even though their consumption can be seen simultaneous. Also, because of the energetic issue, the maltotriose consumption is almost neglected compared to the glucose and maltose and most brewers consider the maltotriose a non-fermentable sugar.
The ordinary different equations for the carbohydrates consumption based on the reaction scheme are given by:

$$\frac{dG}{dt} = -\mu_1 \, X \qquad (1.14) \qquad \frac{dM}{dt} = -\mu_2 \, X \qquad (1.15) \qquad \frac{dN}{dt} = -\mu_3 \, X \qquad (1.16)$$

The consumption rate of maltose (1.18) and maltotriose (1.19) presents a consumption part and an inhibition part. The maltose one (1.18) is inhibited by glucose while the maltotriose consumption rate (1.19) is inhibited by glucose and maltose. The glucose consumption rate (1.17) does not present this inhibition part because it is the priority sugar.

$$\mu_1 = \frac{\mu_G \, G}{K_G + G} \tag{1.17}$$

$$\mu_2 = \frac{\mu_M \, M}{K_M + M} \, \frac{K'_G}{K'_G + G} \tag{1.18}$$

$$\mu_3 = \frac{\mu_N \; N}{K_N + N} \; \frac{K'_G}{K'_G + G} \; \frac{K'_M}{K'_M + M} \tag{1.19}$$

Their maximum reaction velocities (1.20), Michaelis constants (1.21) and inhibition constants (1.22 are modelled using the Arrhenius equation [25], so they represent the temperature dependence of the reaction rates.

$$\mu_{\mathrm{i}} = \mu_{\mathrm{i0}} \; e^{-\frac{\mathrm{E}_{\mu\,\mathrm{i}}}{RT}} \;\; , \;\; i = G, M, N \tag{1.20}$$

$$K_{\mathrm{i}} = K_{\mathrm{i0}} \; e^{-\frac{E_{\mathrm{Ki}}}{RT}} \;\; , \;\; i = G, M, N \tag{1.21}$$

$$K'_{\mathrm{i}} = K'_{\mathrm{i0}} \; e^{-\frac{E'_{\mathrm{Ki}}}{RT}} \;\; , \;\; i = G, M \tag{1.22}$$

The biomass ODE (Equation 1.23) also follows the Stoichiometric Equation 1.1 and the reaction scheme. Its specific growth rate (1.24) has two parts, the growth and the inhibition. The growth part is a sum of the consumption rates of each sugar multiplied by the yields of each sugar with respect to biomass.

$$\frac{dX}{dt} = \mu_{\mathrm{X}} \; X \tag{1.23}$$

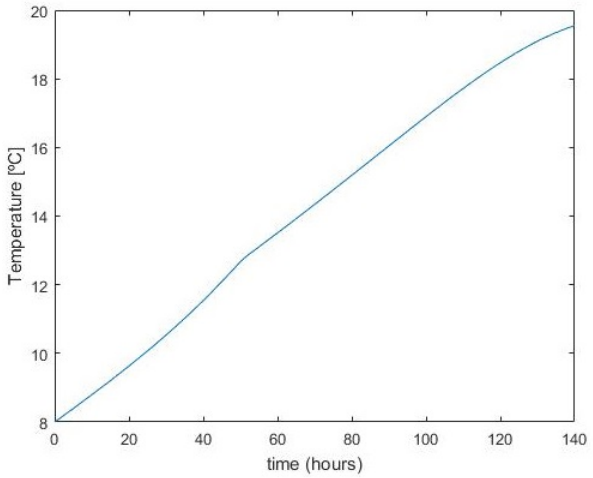
$$\mu_{\mathrm{X}} = (Y_{\mathrm{XG}} \; \mu_1 + Y_{\mathrm{XM}} \; \mu_2 + Y_{\mathrm{XN}} \; \mu_3) \; \frac{K_{\mathrm{X}}}{K_{\mathrm{X}} + (X - X_0)^2} \tag{1.24}$$

Different from the other state variables the Ethanol concentration (Equation 1.25) is given by an algebraic relationship that was derived from its ODEs simplification. So the assumption is that the ethanol production is inversely proportional to the amount of the sugar consumption.

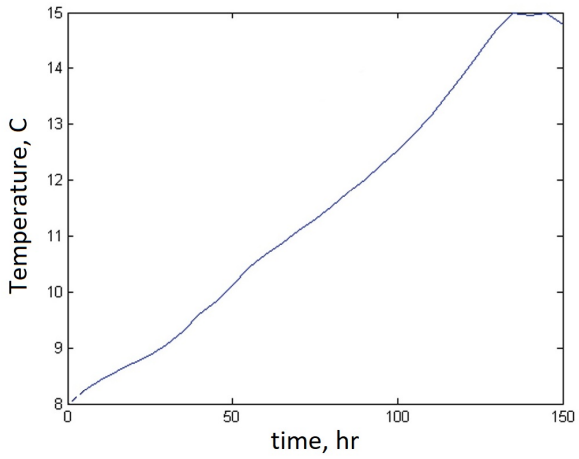
$$E = E_0 + Y_{\mathrm{EG}}(G_0 - G) + Y_{\mathrm{EM}}(M_0 - M) + Y_{\mathrm{EN}}(N_0 - N) \tag{1.25}$$

To find the temperature of the system it is necessary to do an energy balance. The temperature evolution over time depends on the heat from the chemical reactions and a controlling term with a cooling capacity.

$$\frac{dT}{dt} = \frac{1}{\rho c_{\mathrm{p}}} \left(\Delta \; \mathrm{H_{FG}} \frac{dG}{dt} + \Delta \; \mathrm{H_{FM}} \frac{dM}{dt} + \Delta \; \mathrm{H_{FN}} \frac{dN}{dt} - u(T - T_{\mathrm{c}}) \right) \tag{1.26}$$



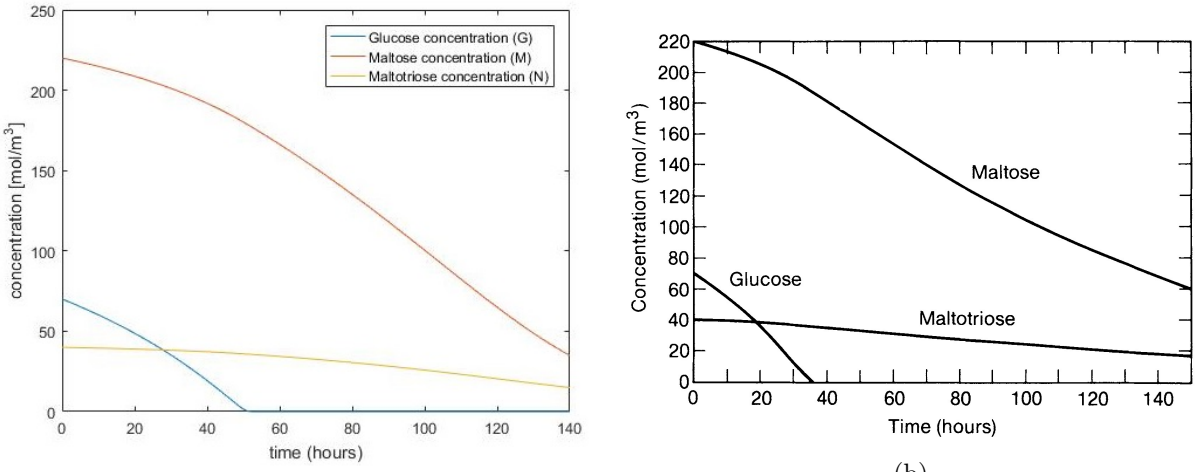
(a)



(b)

Figure 1.3: Simulation of Temperature for Ramirez & Gee: (a) Model; (b) Optimal temperature profile using an optimal flavour objective [26].

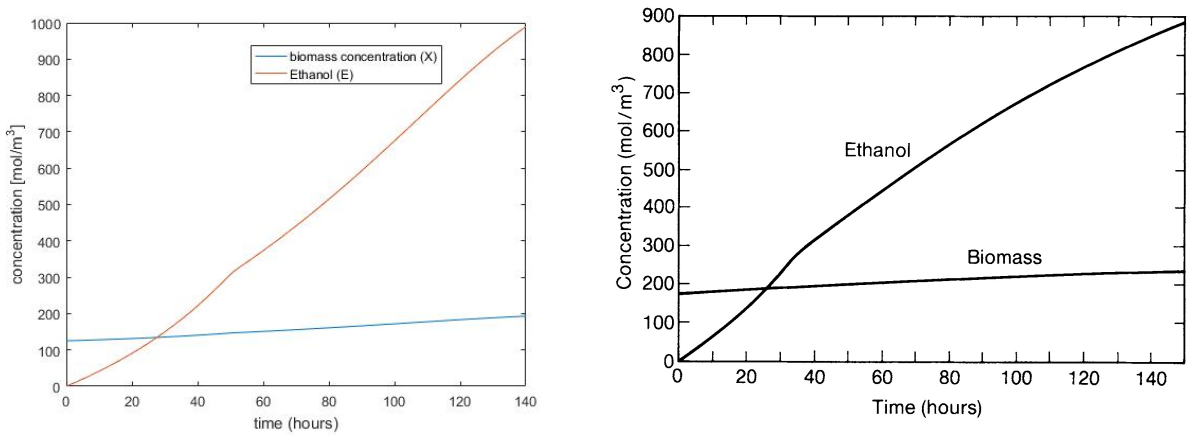
The simulations are implemented in MATLAB® script using a non-stiff differential equation solver (ode45 [23]) function and the parameters were taken from articles [11], [13], [14] and [26]. Because of some conflicting parameter values, it was chosen a main article and the others would be serving as support papers, and the main article chosen is [11].
In [11] Ramirez & Gee discuss the optimal temperature control for batch beer fermentation systems. Since it was not implemented, the control and the temperature coolant are only constants. It is inappropriate to compare the temperature profile for both cases. Also, temperature has an effect on the kinetics so all results are impacted by its profile and changes are expected. In [26] there is a temperature profile (Figure 1.3b) that follows similar situations to the ones encountered in the model (Figure 1.3a), so comparisons could be made with the system following this temperature profile, if [26] presented the other components and their curves for this particular profile, which it does not.
For the sugar consumption curves, the model behaves as expected. The glucose concentration drops very fast because it is the preferred fermentable sugar of yeast, since it is the easiest to break. The second one is maltose, which is the one with larger concentration. Since maltose is harder to break, its concentration decreases slower. The maltotriose, which is almost a non-fermentable sugar, has a small decrease of its concentration. Comparing the model 1.4a to the articles 1.4b, there are some expected differences, due to some different parameters, but the behaviours are similar and can be considered acceptable.



(a) (b)

Figure 1.4: Simulation of sugars for Ramirez & Gee: (a) Model; (b) Simulation of optimal fermentation sugar profile [11].

The exhaustion of glucose concentration is supposed to be noticeable in the profile of the ethanol production and its growth should be almost linear due to the temperature profile and the fact that there is still sugar in the wort. If the concentration of sugars in the wort finished, ethanol concentration would be constant, meaning that its curve would go to a steady state position.
The biomass production needs to be smaller than the other productions, but it should grow. If it stops growing, or the curve falls, it means that the cells are dying or something is wrong with the fermentation.
Comparing the model profile, Figure 1.5a, with the article, Figure 1.5b, ethanol seems to grow more than expected and its curve looks more pointed, while in the reference it looks more attenuated. The reason should be because the two system work with different temperature profiles. In both figures, around 50h, it is possible to see the bump of the glucose concentration end.
For the biomass concentration, one of the parameters needed to be stipulated, which can explain



(a) (b)

Figure 1.5: Simulation of ethanol and biomass for Ramirez & Gee: (a) Model; (b) Simulation of optimal fermentation ethanol and biomass profile [11].

the different shapes of the curves. Also Ramirez & Gee in [11] used two initial values for biomass concentration, found in a table of the parameters, which was the one used in the model, and the one presented in the Figure 1.5b, and that is the reason for the different initial values.

1.4.3 Andrés-Toro *et al.* Model

As explained before, this kinetic model takes into consideration five state variables: biomass (X), substrate (C_s), ethanol (C_e), diacetyl (C_{dy}) and ethyl acetate (C_{ea}). Since diacethyl and ethyl acetate are by-products, their model development won't be studied. The model summary can be seen in Appendix A in Subsection A.3.3.

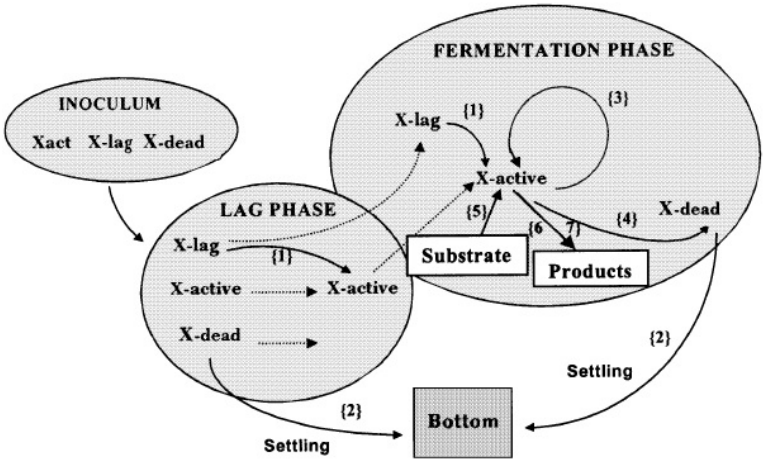


Figure 1.6: Process scheme considered in the kinetic model [15].

This model takes into consideration the behaviour of the biomass. Its behaviour can be seen in Figure 1.6. They divide the fermentation process into two phases (lag and fermentation) and three different types of yeast cells are considered: lag (X_{lag}), active (X_{act}) and dead (X_{dea}). Bottom is literally the bottom of the bioreactor and inoculum is the biomass before the start of the lag phase.

Also, a temperature dependency is modelled (1.27). This dependency is calculated using the Arrhenius equation and introduced in the parameters inside the specific growth rates.

$$\mu_{i_0} = A\ e^{B/RT} \tag{1.27}$$

The inoculation is the act of pouring the yeast into the wort, so one could say that the inoculum "phase" of Figure 1.6 is the initial conditions of the biomass (in this case, it was considered that the inoculum biomass (X_{inc}) has 50% of dead cells, 48% of lag cells and 2% of active cells).

$$X_{act}(0) + X_{lag}(0) = 0.5\ X_{inc}(0),\ for\ t\ <\ t_{lag} \tag{1.28}$$

Right after the inoculum "phase" is the lag phase. Two behaviours are modelled in this phase. The yeast becomes suspended (1.29) in the wort and the dead cells slowly goes down (in Figure 1.6 it is represented by the "Settling" arrow). This behaviour is also modelled with a settling down rate (1.31). The suspended cells movement is modelled by Equation 1.30. Also in the lag phase, part of the lag yeast becomes active. As seen in Figure 1.6 this reaction is represented by the number {1} arrow being the second modelled behaviour (1.32).

$$X_{sus} = X_{act}(t) + X_{lag}(t) + X_{dea}(t) \tag{1.29}$$

$$\frac{dX_{sus}(t)}{dt} = -\mu_{SD}(X_{sus}(t) - 0.5\ X_{inc}),\ for\ t\ <\ t_{lag} \tag{1.30}$$

$$\mu_{SD} = \frac{0.5\ \mu_{SD_0}\ C_{S_0}}{0.5\ C_{S_0} + C_e(t)} \tag{1.31}$$

$$\frac{dX_{act}(t)}{dt} = \mu_{lag}\ X_{lag}(t) = \mu_L(0.5\ X_{inc} - X_{act}(t)),\ for\ t\ <\ t_{lag} \tag{1.32}$$

In the fermentation phase the rest of the lag yeast will continue their activation (reaction 1 in Figure 1.6), the dead yeast will settle down (reaction 2 in Figure 1.6) and the active yeast will produce new biomass (reaction 3 in Figure 1.6), produce products like ethanol and by-products (reactions 6 and 7 in Figure 1.6) and part of it will die (reaction 4 in Figure 1.6). The behaviour of the active cells is modelled by Equation 1.33 and its growth rate by Equation 1.34.

$$\frac{dX_{act}(t)}{dt} = \mu_x\ X_{act}(t) + \mu_L\ X_{lag}(t) - \mu_{DT}\ X_{act}(t),\ for\ t\ >\ t_{lag} \tag{1.33}$$

$$\mu_x = \frac{\mu_{x_0}\ C_s(t)}{k_x + C_e(t)} \tag{1.34}$$

The consumption of substrate (1.35) in the fermentation phase has its specific consumption rate (1.36) modelled following the Michaelis-Menten function [27].

$$\frac{dC_s(t)}{dt} = -\mu_s\ X_{act}(t) \tag{1.35}$$

$$\mu_s = \frac{\mu_{s_0}\ C_s(t)}{k_s + C_s(t)} \tag{1.36}$$

There is also the production of ethanol (1.37) in the fermentation phase. Its production function (reaction 6 or 7 in Figure 1.6) has two parts, inhibition and production. The production rate is modelled by the Equation 1.38 and the inhibition part by Equation 1.39.

$$\frac{dC_e(t)}{dt} = f\ \mu_e\ X_{act}(t) \tag{1.37}$$

$$\mu_e = \frac{\mu_{e_0}\ C_s(t)}{k_e + C_s(t)} \tag{1.38}$$

$$f = 1 - \frac{C_e(t)}{0.5\ C_{s_0}} \tag{1.39}$$

To simulate the Andrés-Toro model the system was implemented in a MATLAB® script using a discretization in time by a forward Euler method and the parameter values were taken from the Article [15].
The Model has three phases: the inoculum which is instantaneous, the lag phase that can take from 3 to 15h right after the inoculation of the yeast and the fermentation phase that can last roughly two to three weeks for most of the yeast. Since the first two phases have no substrate consumption and the lag phase is only for cells activation and movement, the most important phase is the fermentation, so the simulation of the model is based only on the last phase.
The temperature profile was taken from an image of [15], and used to calculate all parameter values to the model. Also, there are some parameters whose values were uncertain. Due to these facts, inaccuracy might occur and some errors are expected.

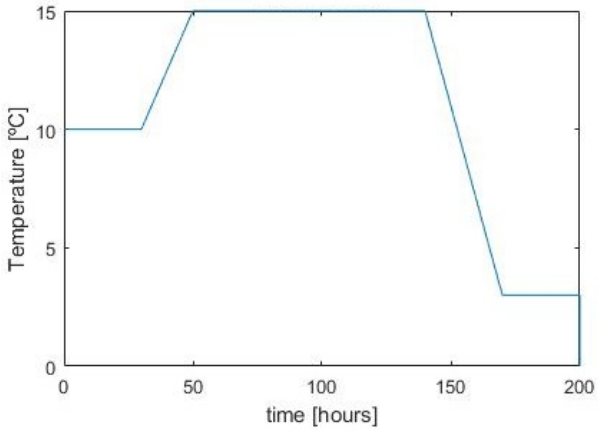
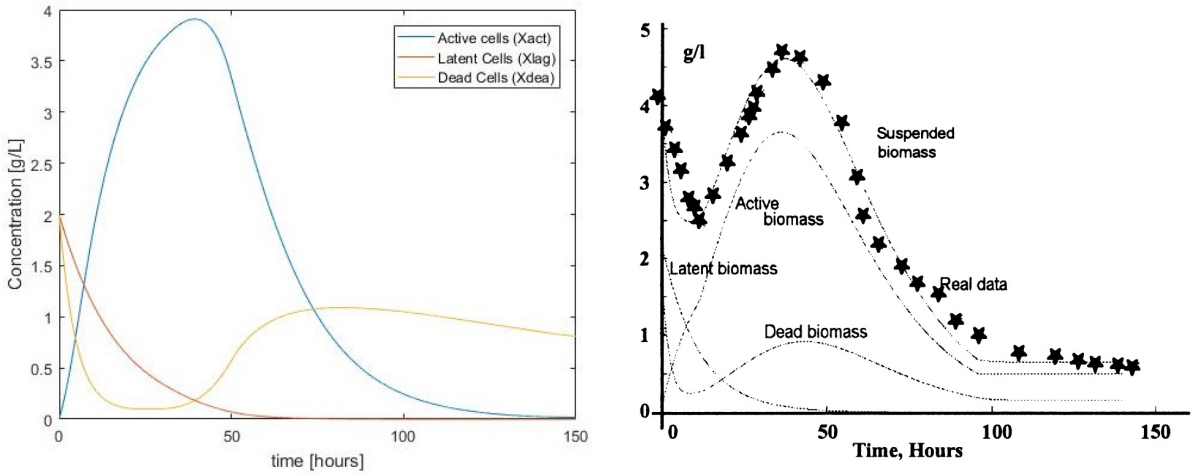


Figure 1.7: Industrial temperature profile

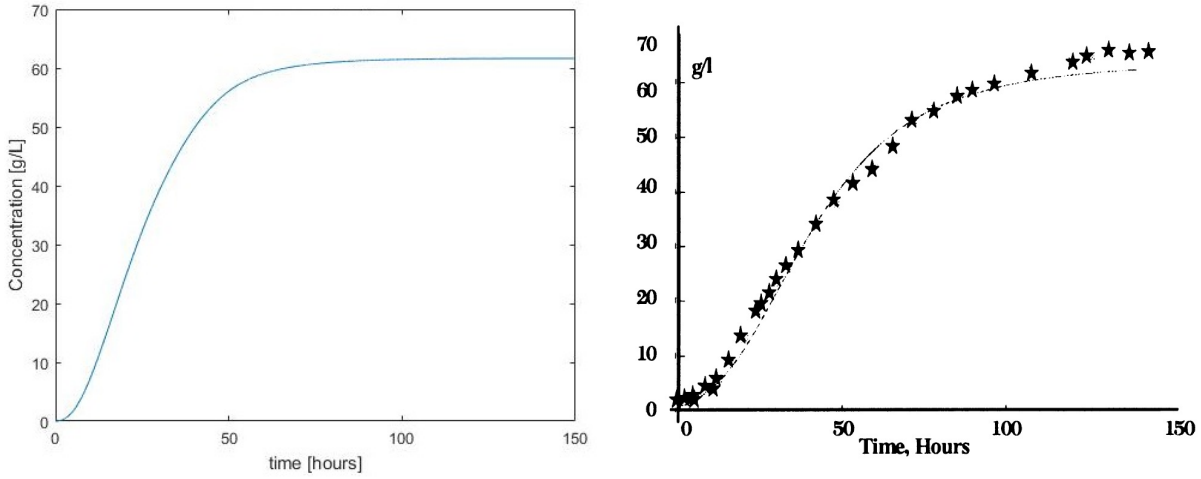
The biomass is divided in active, latent (lag) and dead cells. Their active cells should grow at the beginning because of activation from the latent cells and own growth. When the sugar concentration vanishes these cells starts to die or to hibernate. The latent cells exist only at the



(a) (b)

Figure 1.8: Simulation of biomass variable for Andrés-Toro: (a) Model; (b) Time courses of the concentration of biomass. Stars: experimental points. Solid line: model prediction [15].

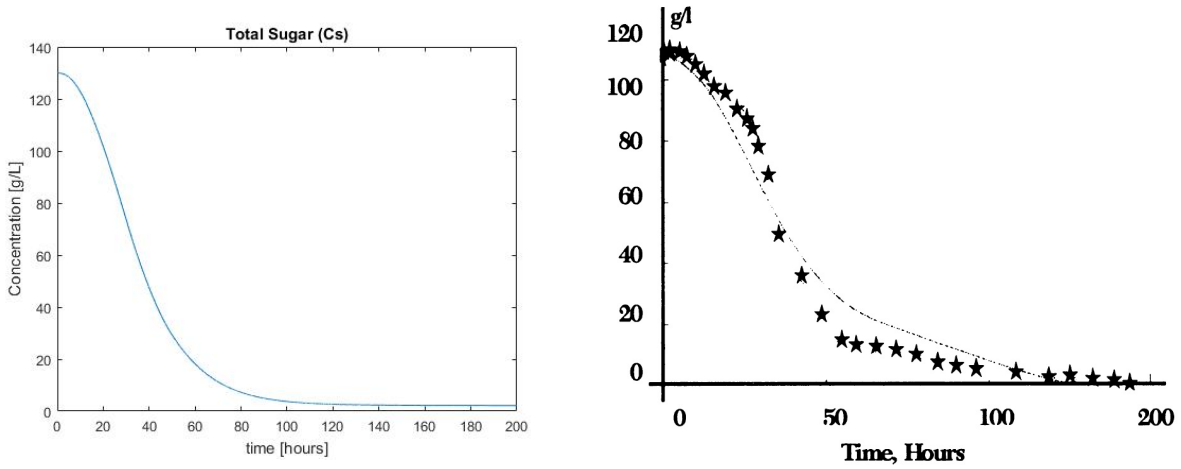
beginning and they are activated into active cells. Dead cells will settle down in the fermenter, so their concentration tends to zero in the wort. At the beginning, dead cells concentration decreases, remnant of the lag phase movement. Afterwards, the concentration grows together with the active cells and slowly settles down again.
Comparing Figures 1.8a with 1.8b, the concentration of active cells and latent cells behave as expected and the curves are similar. The curves for dead cells are different especially at the first valley between 0 and 50h. This disparity is probably one of the errors expected. Fortunately dead cells is not the most important variable for the biomass, which is the active cells, and it does not affect the other variables.
Ethanol concentration is directly related to the actions of the active cells since it is a product of the fermentation of this cells in the search for energy. So the curve should have a slow slope at the beginning. Then an almost linear slope in the middle and a plateau at the end of the fermentation.



(a) (b)

Figure 1.9: Simulation of ethanol variable for Andrés-Toro: (a) Model; (b) Time courses of the concentration of ethanol. Stars: experimental points. Solid line: model prediction [15].

The curves of Figures 1.9a and 1.9b have different slopes, with the curve of 1.9a been faster and tending to a plateau around 60h while the curve of 1.9b tends to a plateau around 140h. The errors caused by the parameter values shouldn't affect significantly the production rate, and this error should be further studied if this model is later used.
The sugar concentration in the wort while in the fermentation phase should gradually decrease. So the sugar curve should at the beginning start slowly being consumed by the active cells, at the middle have an almost linear shape and in the end tend to convergence to zero or close to it, if the fermentation is complete.
Comparing both curves of the Figures 1.10a and 1.10b they have the same bell shape, rate and timing. The simulation curve 1.10ahas small errors but can be consider acceptable and neglectable.



(a) (b)

Figure 1.10: Simulation of total sugars variable for Andrés-Toro: (a) Model; (b) Time courses of the concentration of total sugars. Stars: experimental points. Solid line: model prediction [15].

1.4.4 Comparison

The models were compared qualitatively. The representation of variables substrate, biomass and ethanol will be compared. Also, the existence of temperature and CO_2 variables in the model, the simplicity of the model and the reliability of it will be taken into account.
Another remark is that all models require identification and validation. Parameters are dependent on brewing operational variables such as yeast type, beer style, scale of the fermenter, operation mode among others. So, a given model should always be re-identified in case of a new application and a comparison between models should always considerate this aspects.

Variables
The substrate consumption was modelled by Ramirez & Gee in the same way as Andrés-Toro. It is a function multiplying a specific growth rate by the biomass concentration, which is the actor behind the consumption. Both maximum reaction velocities and Michaelis constants are temperature related and their difference is the fact that Ramirez & Gee separate the sugars.
Balling based model, on the other hand, models the substrate consumption different from the other two. The consumption is the inverse of the sum of the growths of biomass and ethanol multiply by their Balling yield with respect to substrate. This is not a common approach for this type of kinetics. This approach can be due to the fact that the Balling based model does not model the substrate consumption but in reality models the specific gravity behaviour.
For the biomass growth, Balling based model and Ramirez & Gee's model have the same structure but totally different specific growth rates and since Andrés-Toro's model is more focused in the behaviour of biomass, its model is very unique.
The specific growth rate in Ramirez & Gee model has a dependency on temperature and it is attached to all of the sugars consumptions. With the Balling based specific growth rates, one can choose between three kinetic models and depending on the choice it can depend only in sugar or also in ethanol.
Andrés-Toro's model is a more elaborated model for biomass, and it must be used if one needs the behaviour of the yeast in the fermenter. For cases where the behaviour of the cells are not important, but its reactions, this model looses part of its purposes due to its complexity.
Ramirez & Gee model's ethanol production is an algebraic linear model from the values of the concentration of the sugars multiplied by their respective yields over ethanol.

Both Balling's and Andrés-Toro's models or ethanol production uses biomass concentration multiplied by ethanol's specific growth rate but Andrés-Toro's model has also multiplied its own concentration and a constant in the function.

Temperature and CO_2

The presence of temperature in a kinetic model of fermentation is debatable due to the fact that most of this systems have temperature control and this variable is considered for most of the time constant. Due to this fact, models like Balling, where temperature is not considered as a variable changing through time, simplifies their signals by taking out the Arrhenius equations with this process being considered acceptable.

Models that takes into consideration temperature as a variable are temperature controlled models. Cases like Andrés-Toro uses controlled temperature profiles, that won't change according to any other variable, while cases like Ramirez & Gee has a temperature signal that varies according to the reactions but it also has a control signal actuating to prevent this temperature to rise or fall, if turned on, maintaining it constant.

In the end, the presence or absence of the temperature in the model depends exclusively on what the model will be used for. For this project, the temperature, at first won't be necessary, since the temperature is considered constant and simplifications needs to be done.

The only model that presents released CO_2 is the Balling based model but it is in its algebraic linear model and is the measurement, which is hard to replicate since there is no data record on the article neither a figure showing its profile or a mathematical model. So comparisons can't be made and none of the models presents CO_2 as a state variable.

Model Complexity and Reliability

The Balling based model is the simplest. One can use its algebraic linear model, that is the simplest this models can get, but if only considering the ODE systems, the Balling model with Monod kinetics is still the simplest due to the fact that it does not consider temperature as a variable. The second simplest model is the Andrés-Toro because it does not give equations of maximum velocity neither the Michaelis constants, instead, it gives parameter values related to a temperature profile already selected. The most complex one is the Ramirez & Gee. With a complex relation with temperature and three types of sugar.

The three models are well studied and their mathematical developments follows similar systems, so they are trustworthy. The reliability must be in the parameter values and the simulations presented in the articles.

In the article of Balling based model the parameters were all given but the simulations weren't. The writers also provided model error values and efficiency coefficients to validate the parameter values. The Ramirez & Gee model did not present all parameter values, so some had to be presumed, but the simulations were well done and discussed. Also, this model had four articles that complemented each other which helped in the implementation. Andrés-Toro's model also did not present all parameter values and they had to be presumed, but the simulations were presented.

Comparing the three models, the Ramirez & Gee model is the most reliable due to its parameters and the fact that its simulations were the ones that one could replicate similar to the articles.

Chapter 2

Case Studies

In the development of this work, it is assumed that the carbon dioxide flow, specific gravity and temperature are available for monitoring purposes. Then, it will be necessary to devise a model where these measurements are associate to the variables of interest such as sugar and ethanol concentrations. For instance, the carbon dioxide flow is directly related to the production of CO_2, the specific gravity is related to the consumption of sugars and the temperature influences in the reaction rates. In this chapter a change of the already displayed models is discussed to fit the project needs and a model is devised.

2.1 Modified Models

Balling Based Model
For the Balling based model, the Aiba kinetics is very interesting because it relates the ethanol poisonous behaviour towards the yeast and it is a very simple model. In [10] there is a simplification of this model where it is applied the CO_2 measurement where the Function 2.1 is created assuming a Gay-Lussac relationship. So, with it, the CO_2 signal is created.

$$S(t) = S_{int} - 0.5157 \times CO_2(t) \tag{2.1}$$

$$CO_2(t) = \frac{S_{int} - S(t)}{0.5157} \tag{2.2}$$

Ramirez & Gee Model
To implement CO_2 into the Ramirez & Gee Model, it is necessary to use the reaction scheme (1.11, 1.12 and 1.13), and unfortunately, this parameters wont have values. Considering that the reaction rate of the biomass is the same for the CO_2 only with a yield multiplication differentiating it, than:

$$\frac{dCO_2}{dt} = (Y_{CO_2G}\ \mu_1 + Y_{CO_2M}\ \mu_2 + Y_{CO_2N}\ \mu_3)\ X \tag{2.3}$$

Or it could be like the ethanol signal, modelled as a linear equation.

$$CO_2 = CO_{20} + Y_{CO_2G}(G_0 - G) + Y_{CO_2M}(M_0 - M) + Y_{CO_2N}(N_0 - N) \tag{2.4}$$

Andrés-Toro *et al.* Model
To add CO_2 production into this model, it must be in the fermentation phase. The active yeast will produce a new product (CO_2) in a new reaction {8} and then, Figure 1.6 will be changed a bit. The new model for the CO_2 signal will be:

$$\frac{dC_{CO_2}(t)}{dt} = \mu_{CO_2}\ X_{act}(t) \qquad (2.5) \qquad\qquad \mu_{CO_2} = \frac{\mu_{CO_{20}}\ C_s(t)}{k_{CO_2} + C_s(t)} \qquad (2.6)$$

2.2 Proposed Model

The representative models, even with their modifications, have some drawbacks, such as uncertain parameters or the presence of more variables than necessary, that hinders their application on this project. Also, as explained before, to apply a model to a new case, re-identification is needed. So, to fit better the necessities of this project and to avoid this issues, a new model is proposed. This devised model needs to present some essential characteristics. The first one is the presence of emitted CO_2 measurement. The second is that this model needs to be a kinetic model that represents a simplification of the catabolic reactions of the fermentation process of beer in a batch mode. At first instance, all the fermentable carbohydrates should be considered of one type and temperature and pressure as constants in time.

Mass Balance
The Equation 2.7 is a catabolic reaction of the fermentation process for yeast based in [24] and it is a simplification of the stoichiometric Equation (1.1) and the enzymatic reactions.

$$k_{\mathrm{S}} S \xrightarrow{\mu_X X} k_{\mathrm{E}} E + k_{\mathrm{CO_2}} CO_2 + k_{\mathrm{X}} X \qquad (2.7)$$

Where S is the consumed substrate, E the produced ethanol, CO_2 the produced carbon dioxide, X is the produced biomass, μ_{X} is the reaction rate and $\mathrm{k_i}$ are the yield coefficients. Dividing all the terms by $\mathrm{k_X}$ the catabolic reaction becomes:

$$\frac{k_{\mathrm{S}}}{k_{\mathrm{X}}} S \xrightarrow{\mu_X X} \frac{k_{\mathrm{E}}}{k_{\mathrm{X}}} E + \frac{k_{\mathrm{CO_2}}}{k_{\mathrm{X}}} CO_2 + X \qquad (2.8)$$

With the catabolic reaction (2.8) it is possible to do the mass balance of the system that will be a system of ODEs.

$$\frac{dS}{dt} = -k_{\mathrm{SX}}\ \mu_{\mathrm{X}} X \qquad (2.9)$$

$$\frac{dX}{dt} = \mu_{\mathrm{X}} X \qquad (2.10)$$

$$\frac{dE}{dt} = k_{\mathrm{EX}}\ \mu_{\mathrm{X}} X \qquad (2.11)$$

$$\frac{dCO_2}{dt} = k_{\mathrm{CO_2X}}\ \mu_{\mathrm{X}} X \qquad (2.12)$$

Where the production rate (Equation 2.13) is modelled using the Monod equation [20] with an inhibitory effect described in [24] using the Herbert's kinetic model. This inhibitory effect is to describe the influence of the ethanol concentration in an ethanolic fermentation process.

$$\mu_{\mathrm{X}} = \mu_{\max} \frac{S}{K_{\mathrm{X}} + S}\ e^{-k_{\mathrm{iE}}\ E} \qquad (2.13)$$

This differential equation system has some important features. One of them is that there is no aerobic fermentation, that can be confirmed by the Crabtree effect discussed in 1.2.1. A summary of this model was created and can be seen in Appendix B in Section B.2.

An important aspect is the discussion that the CO_2 presented here is the produced CO_2 and this molecule concentration has two fractions a dissolved CO_2 in the wort and a released CO_2, since we are measuring the carbon dioxide transfer rate from liquid to gas phase, it is necessary to find a relationship between them all.

CO_2 production

Following [18], the produced CO_2 is the sum of the released CO_2 and the dissolved one. Since both have an interaction, which is the part of dissolved CO_2 that is released, this transfer rate must be included in the model of their production rate.

$$CO_2 = Y_{C_d/CO_2} C_d + Y_{C_e/CO_2} C_e \tag{2.14}$$

$$\frac{dC_d}{dt} = Y_{C_d/CO_2} \frac{dCO_2}{dt} - CTR \tag{2.15}$$

$$\frac{dC_e}{dt} = Y_{C_e/CO_2} \frac{dCO_2}{dt} + CTR \tag{2.16}$$

Where CTR represents the carbon dioxide transfer rate from the wort to the air, C_e is the emitted carbon dioxide (The CO_2 in the air), C_d is the dissolved carbon dioxide, Y_{C_d/CO_2} and Y_{C_e/CO_2} are yield coefficients and $Y_{C_d/CO_2} + Y_{C_e/CO_2} = 1$. A validation of this equations was done and can be seen in Appendix B in Section B.1.

The Carbon Dioxide Transfer Rate (CTR) is one of the signals that can be measured so it is very important to find an equation which represents it over the other state variables. To find this relation one will rely on the assumption that the dissolved CO_2 is constant during time because it reaches very fast the saturation. So, the Equation 2.15 is equal to zero.

$$\frac{dC_d}{dt} = Y_{C_d/CO_2} \frac{dCO_2}{dt} - CTR = 0 \tag{2.17}$$

$$CTR = Y_{C_d/CO_2} \frac{dCO_2}{dt} \tag{2.18}$$

The consequence of the assumption of the fast dynamic of the gases is that the CO_2 produced will go directly to the air, meaning that the production of CO_2 will be equal to the production of C_e. To check if this effect happens, one will introduce the Equation 2.18 into Equation 2.16.

$$\frac{dC_e}{dt} = Y_{C_e/CO_2} \frac{dCO_2}{dt} + Y_{C_d/CO_2} \frac{dCO_2}{dt} = \underbrace{(Y_{C_e/CO_2} + Y_{C_d/CO_2})}_{=1 \text{ (following eq.??)}} \frac{dCO_2}{dt} \tag{2.19}$$

$$\frac{dCO_2}{dt} = \frac{dC_e}{dt} \tag{2.20}$$

To finish the calculation of CTR one must extract the derivative part of the Equation 2.18 by using Equation 2.12.

$$CTR = Y_{C_d/CO_2}\ k_{CO_2X}\ \mu_X X \tag{2.21}$$

To conclude, it is important to remark that, following [18], the CO_2 produced is only constant if the system is isobaric and isothermal. Even though that, in this simplified system one will consider that the evolution of the gas is much faster than the evolution of temperature so the temperature influences will be neglected. The pressure will also be neglected because most of the bioreactor systems for beer have a pressure control, which does not give a perfect constant pressure, but in the case of this model it is assumed that it is an isobaric system.

2.3 Observability Analysis

Observability is a system characteristic, introduced in control theory by R. E. Kalman in early 60's which indicates that the system trajectory can be reconstructed from a set of measurements. For instance, when developing a monitoring system the observabiity analysis is particularly interesting to show what are the needed measurements to be able to estimate non-measured state variables. Consider the following state space realization of a linear unforced system:

$$\begin{cases} \dot{x}(t) = Ax(t),\ x(0) = x_0 \\ y(t) = Cx(t) \end{cases} \tag{2.22}$$

Where $x \in \mathbb{R}^n$ is the state vector, x_0 the initial condition, $y \in \mathbb{R}^q$ is the measurement output, A is the state matrix (with dim[A(.)] = n × n) and C is the output matrix (with dim[C(.)] = q × n). Hence, the System (2.22) is observable if an unknown initial condition x_0 can be determined from the measurement $y(t)$ over a finite period of time. This property can be evaluated by means of the observability matrix O as defined in Equation 2.23. The rank of the observability matrix indicates how many elements of x_0 can be determined from the measurements. For instance, the whole n elements of x_0 can be determined if the rank$\{O\} = n$.

$$O = \begin{bmatrix} C \\ CA \\ CA^2 \\ \vdots \\ CA^{n-1} \end{bmatrix} \tag{2.23}$$

However, the beer fermentation process is described by a set of non-linear ordinary differential equations and the concept of observability of linear systems cannot be directly applied. Unfortunately, the local observability, defined in 2.23, is not sufficient to ensure global observability of a bioreactor non-linear system. Moreover, in the proposed setting, one needs to check the observability because there is only the measures of temperature, specific gravity and evolved CO_2 rate and one wants to monitor all the state variables that is substrate, ethanol and biomass. A natural extension of the definition of observability for non-linear dynamical systems is to evaluate the observability along the system trajectory.
Observability along the state trajectories can be determined and discussed. The evolution of the rank$\{O\}$ therefore becomes a necessary but not sufficient observability condition. The idea is to define an analytical expression of O allowing one to determine the evolution of its rank with respect to the state and parameter values for a specific output configuration. In order to achieve this, it is calculated for several operating points along the trajectory, providing a good indication of global observability.

2.3.1 An Analytical Discussion of the Balling Based Model

Balling based model, described in Section 1.4, has three state variables which are biomass (X), ethanol (P) and substrate (S) and the functions f_1 (2.24), f_2 (2.25) and f_3 (2.26) are the derivatives over time of the biomass ($\frac{dX}{dt}$), ethanol ($\frac{dE}{dt}$) and substract ($\frac{dS}{dt}$) concentrations respectively.

$$f_1 = \mu(t)X(t) \tag{2.24}$$

$$f_2 = q(t)X(t) \tag{2.25}$$

$$f_3 = -\frac{1}{Y_{X/S}}\frac{dX}{dt} - \frac{1}{Y_{E/S}}\frac{dE}{dt} \tag{2.26}$$

Assuming a Monod reaction rate, the linearized state matrix (Jacobian) will be:

$$A = \begin{bmatrix} \frac{\partial f_1}{\partial X} & \frac{\partial f_1}{\partial P} & \frac{\partial f_1}{\partial S} \\ \frac{\partial f_2}{\partial X} & \frac{\partial f_2}{\partial P} & \frac{\partial f_2}{\partial S} \\ \frac{\partial f_3}{\partial X} & \frac{\partial f_3}{\partial P} & \frac{\partial f_3}{\partial S} \end{bmatrix} = \begin{bmatrix} \frac{S\mu_{max}}{K_{sx}+S} & 0 & \frac{X\mu_{max}K_{sx}}{(K_{sx}+S)^2} \\ \frac{Sq_{max}}{K_{sp}+S} & 0 & \frac{Xq_{max}K_{sp}}{(K_{sp}+S)^2} \\ \frac{-S\mu_{max}}{Y_{xs}(K_{sx}+S)} - \frac{Sq_{max}}{Y_{ps}(K_{sp}+S)} & 0 & -\frac{X\mu_{max}K_{sx}}{Y_{xs}(K_{sx}+S)^2} - \frac{Xq_{max}K_{sp}}{Y_{ps}(K_{sp}+S)^2} \end{bmatrix} \tag{2.27}$$

By observing the Jacobian matrix 2.27 it is clear the appearance of the column of zeros. This Column represents that if the second state variable is not measured, the system is not observable. For the rest of the state variables, it is necessary to continue researching.
With the state matrix it is possible to calculate the observability Matrix 2.28 and its rank with respect to the different output matrix. It was chosen to verify the observability with only one measured output per time, so there will be three cases of output matrix. And for detailed elements of the observability matrices the reader is encourage to seek Appendix C in Section C.2.

$$O = \begin{bmatrix} C \\ CA \\ CA^2 \end{bmatrix} \tag{2.28}$$

If it is assumed that only the biomass concentration is measurable, the output matrix will be $C = [1\ 0\ 0]$ and the the following observability matrix is obtained:

$$O = \begin{bmatrix} 1 & 0 & 0 \\ \frac{\mu_{max}S}{S+K_{sx}} & 0 & \frac{\mu_{max}K_{sx}X}{(S+K_{sx})^2} \\ CA_1^2(S,X) & 0 & CA_3^2(S,X) \end{bmatrix} \tag{2.29}$$

In this particular case, the rank of the observability matrix O as defined in Matrix 2.29 is equal to 2 for either a non-zero biomass or substrate concentration. This is due to the fact that there is a full column of zeros. Thus, in this case, even changing some model parameters, the second state of this particular system model will not be observable.

On the hand, if it is assumed that the ethanol concentration is measurable, the output matrix will be $C = [0\ 1\ 0]$ and the following analysis is carried out:

$$O = \begin{bmatrix} 0 & 1 & 0 \\ O_{21}(S) & 0 & O_{23}(S,X) \\ O_{31}(S,X) & 0 & O_{33}(S,X) \end{bmatrix} \tag{2.30}$$

In this case, there is no full-zero column, so the rank could be 3 considering some specific values for the parameters. The next step is to define the limitations of the parameter values. And to find these limitations, the matrix is transformed into its echelon form by using some Gaussian elimination. In its echelon form, it is possible to calculate the rank of the matrix. All the mathematical development can be found in Appendix C in Section C.3. In this case the system will be observable (rank equals to 3) if and only if the last operation of the echelon transformation is impossible (Equation C.19), so the test of observability is given by Equation 2.31.

$$\frac{O_{33}O_{21} - O_{23}O_{31}}{O_{21}} \neq 0 \tag{2.31}$$

After some mathematical manipulations (All the mathematical reasoning can be seen in the Appendix C in Section C.4), the analysis ends up by checking the behaviour of the system in

the case where S or X are equal to zero since all other parameters are constants and positive. Eventually, it may be concluded that the observability test will fail if and only if S and/or X is equal to zero. $S = 0$ means that the specific gravity is zero. This, in degrees Plato means that the solution has the same density as water, which is physically impossible. For $X = 0$, it means that the reaction never started. The conclusion is that this system is likely to be observable when ethanol is measured and so *C=[0 1 0]*.

Similarly of the latter analysis, if only the substrate is measured, the output matrix is given by $C = [0\ 0\ 1]$ and the following analysis is derived:

$$O = \begin{bmatrix} 0 & 0 & 1 \\ \frac{-S\mu_{max}}{Y_{xs}(K_{sx}+S)} - \frac{Sq_{max}}{Y_{ps}(K_{sp}+S)} & 0 & -\frac{X\mu_{max}K_{sx}}{Y_{xs}(K_{sx}+S)^2} - \frac{Xq_{max}K_{sp}}{Y_{ps}(K_{sp}+S)^2} \\ CA_1^2(S,X) & 0 & CA_2^2(S,X) \end{bmatrix} \tag{2.32}$$

Same case as for the observability test in the case of only measuring biomass, the rank of the observability matrix O (2.32) will always be maximum 2.

A numerical approach for this analysis was also made and can be seen in Appendix C in Section C.5.

2.3.2 Analytical Analysis of the Proposed Model

The model proposed in this master thesis has three state variables. The state variables are substrate (S), ethanol (E) and biomass (X) and the functions f_1 (2.33), f_2 (2.34) and f_3 (2.35) are the derivatives over time of the their respective concentrations.

$$f_1 = -k_{SX}\ \mu_{max}\frac{S}{K_X + S}\ e^{-k_{iE}\ E}X \tag{2.33}$$

$$f_2 = k_{EX}\ \mu_{max}\frac{S}{K_X + S}\ e^{-k_{iE}\ E}X \tag{2.34}$$

$$f_3 = \mu_{max}\frac{S}{K_X + S}\ e^{-k_{iE}\ E}X \tag{2.35}$$

Calculating the linearized state matrix $A(x) = \dfrac{\partial f(x)}{\partial x}$.

$$A = \begin{bmatrix} \frac{\partial f_1}{\partial S} & \frac{\partial f_1}{\partial E} & \frac{\partial f_1}{\partial X} \\ \frac{\partial f_2}{\partial S} & \frac{\partial f_2}{\partial E} & \frac{\partial f_2}{\partial X} \\ \frac{\partial f_3}{\partial S} & \frac{\partial f_3}{\partial E} & \frac{\partial f_3}{\partial X} \end{bmatrix} \tag{2.36}$$

$$A = \begin{bmatrix} -\frac{k_{SX}\ \mu_{max}K_X e^{-k_{iE}\ E}X}{(K_X+S)^2} & k_{SX}\mu_{max}\frac{S}{K_X+S}\ k_{iE}\ e^{-k_{iE}\ E}X & -k_{SX}\mu_{max}\frac{S}{K_X+S}\ e^{-k_{iE}\ E} \\ \frac{k_{EX}\mu_{max}K_X e^{-k_{iE}\ E}X}{(K_X+S)^2} & -k_{EX}\mu_{max}\frac{S}{K_X+S}\ k_{iE}\ e^{-k_{iE}\ E}X & k_{EX}\mu_{max}\frac{S}{K_X+S}\ e^{-k_{iE}\ E} \\ \frac{\mu_{max}K_X e^{-k_{iE}\ E}X}{(K_X+S)^2} & -\mu_{max}\frac{S}{K_X+S}\ k_{iE}\ e^{-k_{iE}\ E}X & \mu_{max}\frac{S}{K_X+S}\ e^{-k_{iE}\ E} \end{bmatrix} \tag{2.37}$$

The C matrix can also be calculated as the A matrix, but instead of calculating the partial derivative of functions f_i , the C matrix is calculated using the functions h_i. So for the calculation it is important to know the measurements.

In the physical system, it is possible to measure temperature, specific gravity and released bubble rate. The specific gravity is directly related to the substrate, so it is considered that the substrate is measured. The bubble rate is related to the CO_2 released rate, which means that it is the CTR. Temperature won't be consider in this model.
So two possibilities will be considered. The first one is the case where both CTR and S are measured and the second case is where only CTR is measured.

$$h_1 = CTR = k_{CTR}\ \mu_{max}\frac{S}{K_X + S}\ e^{-k_{iE}\ E}X \tag{2.38}$$

$$h_2 = S \tag{2.39}$$

Where $k_{CTR} = Y_{C_d/CO_2}\ k_{CO_2X}$.

For CTR and S measured, the C matrix is calculated as $C_1 = \begin{bmatrix} \frac{\partial h_1}{\partial S} & \frac{\partial h_1}{\partial E} & \frac{\partial h_1}{\partial X} \\ 1 & 0 & 0 \end{bmatrix}$ (2.40). And for the case where Only CTR is measured C matrix follows $C_2 = \begin{bmatrix} \frac{\partial h_1}{\partial S} & \frac{\partial h_1}{\partial E} & \frac{\partial h_1}{\partial X} \end{bmatrix}$ (2.41).

$$C_1 = \begin{bmatrix} \frac{k_{CTR}\ \mu_{max}K_X e^{-k_{iE}\ E}X}{(K_X+S)^2} & -k_{CTR}\mu_{max}\frac{S}{K_X+S}\ k_{iE}\ e^{-k_{iE}\ E}X & k_{CTR}\mu_{max}\frac{S}{K_X+S}\ e^{-k_{iE}\ E} \\ 1 & 0 & 0 \end{bmatrix} \tag{2.40}$$

$$C_2 = \begin{bmatrix} \frac{k_{CTR}\ \mu_{max}K_X e^{-k_{iE}\ E}X}{(K_X+S)^2} & -k_{CTR}\mu_{max}\frac{S}{K_X+S}\ k_{iE}\ e^{-k_{iE}\ E}X & k_{CTR}\mu_{max}\frac{S}{K_X+S}\ e^{-k_{iE}\ E} \end{bmatrix} \tag{2.41}$$

2.3.2.1 Observability Test

To do the observability test, first the observability matrix (2.42) needs to be calculated. Then a research of the boundary conditions is made by manipulating the observability matrix form and analysing the its rank. And to calculate the rank of O it is necessary to reduce the matrix to its echelon form using the Gaussian elimination. The mathematical procedures to reduce the observability matrix can be found in Appendix C in Section C.7 with the echelon form for both observability matrices.

$$O = \begin{bmatrix} C \\ CA \\ CA^2 \end{bmatrix} \tag{2.42}$$

CTR and S measured
The O matrix will be:

$$O = \begin{bmatrix} \frac{k_{CTR}\ \mu_{max}K_X e^{-k_{iE}\ E}X}{(K_X+S)^2} & -k_{CTR}\mu_{max}\frac{S}{K_X+S}\ k_{iE}\ e^{-k_{iE}\ E}X & k_{CTR}\mu_{max}\frac{S}{K_X+S}\ e^{-k_{iE}\ E} \\ 1 & 0 & 0 \\ CA_{11} & CA_{12} & CA_{13} \\ -\frac{k_{SX}\ \mu_{max}K_X e^{-k_{iE}\ E}X}{(K_X+S)^2} & k_{SX}\mu_{max}\frac{S}{K_X+S}\ k_{iE}\ e^{-k_{iE}\ E}X & -k_{SX}\mu_{max}\frac{S}{K_X+S}\ e^{-k_{iE}\ E} \\ CA_{11}^2 & CA_{12}^2 & CA_{13}^2 \\ CA_{21}^2 & CA_{22}^2 & CA_{23}^2 \end{bmatrix} \tag{2.43}$$

All the values of the matrix CA and CA^2 terms are given in the Appendix C in Section C.6. And to simplify the visualization of the observability matrix, it was chosen to do a change of parameters.

$$\alpha_1(S) = K_X + S \tag{2.44}$$

$$\alpha_2(E) = \mu_{max}\ e^{-k_{iE}\ E} \tag{2.45}$$

$$\alpha_3(S,X) = \frac{k_{SX}\ K_X\ X}{\alpha_1} + (k_{iE}\ k_{EX}X - 1)S \tag{2.46}$$

$$\alpha_4(S,X) = \frac{k_{SX}\ K_X\ X}{\alpha_1} - (k_{iE}\ k_{EX}X + 1)S \tag{2.47}$$

$$\alpha_5(S,X) = \frac{k_{SX}\ K_X\ X}{\alpha_1} - (k_{iE}\ k_{EX}\ X\ S + 1)S \tag{2.48}$$

$$\alpha_6(S,X) = \frac{k_{SX}}{\alpha_1} - (k_{EX}\ k_{iE}\ X - 1)S \tag{2.49}$$

And so, the observability matrix will be:

$$O = \begin{bmatrix} \frac{k_{CTR}\ K_X \alpha_2\ X}{\alpha_1^2} & -\frac{k_{CTR}\ k_{iE}\ \alpha_2\ X\ S}{\alpha_1} & \frac{k_{CTR}\ \alpha_2 S}{\alpha_1} \\ 1 & 0 & 0 \\ \frac{k_{CTR}\ K_X\ \alpha_2^2\ \alpha_3\ X}{\alpha_1^3} & \frac{k_{CTR}\ k_{iE}\ \alpha_2^2 \alpha_3\ X\ S}{\alpha_1^2} & \frac{k_{CTR}\ \alpha_2^2\ \alpha_3\ S}{\alpha_1^2} \\ -\frac{k_{SX}\ K_X \alpha_2\ X}{\alpha_1^2} & \frac{k_{SX}\ k_{iE}\ \alpha_2\ X\ S}{\alpha_1} & -\frac{k_{SX}\ \alpha_2\ S}{\alpha_1} \\ \frac{k_{CTR}\ K_X\ \alpha_2^3\ \alpha_3\ \alpha_4\ X}{\alpha_1^4} & \frac{k_{CTR}\ k_{iE}\ \alpha_2^3\ \alpha_3\ \alpha_5\ X\ S}{\alpha_1^3} & \frac{k_{CTR}\ \alpha_2^3\ \alpha_3\ \alpha_5\ S}{\alpha_1^3} \\ \frac{k_{SX}(\alpha_2\ K_X\ X)^2 \alpha_6}{\alpha_1^3} & \frac{k_{iE}\ k_{SX}\alpha_2^2 \alpha_3\ X\ S}{\alpha_1^2} & \frac{k_{SX}\alpha_2^2\ \alpha_3\ S}{\alpha_1^2} \end{bmatrix} = \begin{bmatrix} O_{11} & O_{12} & O_{13} \\ 1 & 0 & 0 \\ O_{31} & O_{32} & O_{33} \\ O_{41} & O_{42} & O_{43} \\ O_{51} & O_{52} & O_{53} \\ O_{61} & O_{62} & O_{63} \end{bmatrix} \tag{2.50}$$

Analysing the mathematical procedures (details in Appendix C in Section C.7), one can say that the only way that the system can have a rank different from 3 is if all the first equations in C.49 are impossible at the same time, which means that $O_{i3} - O_{i2} \times \frac{O_{13}}{O_{12}} = 0$ where $i = 3, 4, 5, 6$.

$$O_{33} - O_{32} \times \frac{O_{13}}{O_{12}} = \frac{2\ k_{CTR}\ \alpha_2^2\ \alpha_3\ S}{\alpha_1^2} = 0 \tag{2.51}$$

$$O_{43} - O_{42} \times \frac{O_{13}}{O_{12}} = 0 \tag{2.52}$$

$$O_{53} - O_{52} \times \frac{O_{13}}{O_{12}} = \frac{2\ k_{CTR}\ \alpha_2^3\ \alpha_3\ \alpha_5\ S}{\alpha_1^3} = 0 \tag{2.53}$$

$$O_{63} - O_{62} \times \frac{O_{13}}{O_{12}} = \frac{2\ k_{SX}\alpha_2^2\ \alpha_3\ S}{\alpha_1^2} = 0 \tag{2.54}$$

After some mathematical manipulation, the Equation 2.52 will be equal to 0 independent of the values of the parameters, with that said, one of the rows of the observability matrix will be fully with zeros. So the verification if the rest of the rows can be fully with zeros must be done with the rest of the equations. For that, the parameters were returned to its original form as shown in Equations 2.55, 2.56 and 2.57.

$$\frac{2\ k_{CTR}\ \mu_{max}^2\ e^{-2k_{iE}E}\left(\frac{k_{SX}\ K_X\ X}{K_X\ X} + (k_{iE}\ k_{EX}X - 1)S\right)S}{(K_X + S)^2} = 0 \tag{2.55}$$

$$2\ k_{CTR}\ \mu_{max}^3\ e^{-3k_{iE}E} \times \frac{\left(\frac{k_{SX}\ K_X\ X}{K_X\ X} + (k_{iE}\ k_{EX}X - 1)S\right)\left(\frac{k_{SX}\ K_X\ X}{\alpha_1} - (k_{iE}\ k_{EX}\ X\ S + 1)S\right)S}{(K_X + S)^3} = 0 \tag{2.56}$$

$$\frac{2\ k_{SX}\ \mu_{max}^2\ e^{-2k_{iE}E}\left(\frac{k_{SX}\ K_X\ X}{K_X\ X} + (k_{iE}\ k_{EX}X - 1)S\right)S}{(K_X + S)^2} = 0 \tag{2.57}$$

Only CTR measured

The O matrix for only measuring CTR is a simplification of the O matrix of when S is also measured. So, some terms of these O matrices are the same, as seen in Equations 2.50 and 2.58.

$$O_b = \begin{bmatrix} \frac{k_{CTR}\ K_X \alpha_2\ X}{\alpha_1^2} & -\frac{k_{CTR}\ k_{iE}\ \alpha_2\ X\ S}{\alpha_1} & \frac{k_{CTR}\ \alpha_2 S}{\alpha_1} \\ \frac{k_{CTR}\ K_X\ \alpha_2^2\ \alpha_3\ X}{\alpha_1^3} & \frac{k_{CTR}\ k_{iE}\ \alpha_2^2 \alpha_3\ X\ S}{\alpha_1^2} & \frac{k_{CTR}\ \alpha_2^2\ \alpha_3\ S}{\alpha_1^2} \\ \frac{k_{CTR}\ K_X\ \alpha_2^3\ \alpha_3\ \alpha_4\ X}{\alpha_1^4} & \frac{k_{CTR}\ k_{iE}\ \alpha_2^3\ \alpha_3\ \alpha_5\ X\ S}{\alpha_1^3} & \frac{k_{CTR}\ \alpha_2^3\ \alpha_3\ \alpha_5\ S}{\alpha_1^3} \end{bmatrix} = \begin{bmatrix} O_{11} & O_{12} & O_{13} \\ O_{31} & O_{32} & O_{33} \\ O_{51} & O_{52} & O_{53} \end{bmatrix} \tag{2.58}$$

Following the same principles of the observability test applied for the case where is measured CTR and S. It is possible to say that the rank of the observability matrix can only be different than three if and only if the Equation 2.59, which is taken from the echelon form, is equal to zero.

$$O_{53} - O_{51}\frac{O_{13}}{O_{11}} - (O_{52} - \frac{O_{51}O_{12}}{O_{11}})(\frac{O_{33} - O_{31}\frac{O_{13}}{O_{11}}}{O_{32} - O_{31}\frac{O_{12}}{O_{11}}}) = 0 \tag{2.59}$$

After some mathematical manipulation, the Equation 2.59 gives:

$$k_{EX}\ S(1 - S)(1 + k_{iE}\ X)(K_X + S) - 2\ k_{SX}\ K_X\ X = 0 \tag{2.60}$$

2.3.2.2 Analysis

For the next part it is important to remember that all constants belongs to the real realm and are positive.

Substrate

In the case where the substrate is equal to zero, the meaning is that there is no substrate in the bioreactor and the reactions would stop. It is a realistic situation, but with the reactions stopped the kinetics will stop too, so this situation is neglected, even though that, it is good to check how the observability goes. And observing the Equations 2.55, 2.56 and 2.57 it is clear that if $S = 0$ they will be true (equal to zero). So the rank of the observability matrix would be 2.
For the Equation 2.60, the observability does not depend on the value of the substrate. If $S = 0$, the Equation 2.60 won't be zero, so the rank is still 3 and the system is still observable. But the observability matrix indicates that if $S = 0$ there will be two columns of zeros so observability matrix would be 1.

Biomass

For the biomass to be zero it means that the yeast all died or there was never yeast on the system. Considering that there will always be a beginning concentration of yeast that will start the fermentation process, the first case, where the yeast dies, is more realistic.
During the process of fermentation, the yeast will procreate and die, but the rate of death is smaller than the born one, so the biomass tends to grow when there is sugar on the system. When the sugar is over, the yeast stops fermenting and most of them will enter on its hibernation

phase. If there is no external interferences like filtering the most or temperature rising over the acceptable for yeast survival, the chances of not having yeast in the wort are rare.
The death rate of the yeast can be caused by some reasons but the most common cases are: high temperature [28], high ethanol concentration [29], high pressure [30] and wort out of the pH acceptable range [31]. So it is good to check the observability in case the yeast dies.
The Equations 2.55, 2.56, 2.57 and 2.60 are false (are not equal to zero), if $X = 0$, meaning that the rank of the observability matrix will always be 3 independent of the value of biomass. Observing the observability matrix, if $X = 0$ for both cases, there will appear two column of zeros indication a rank of 1.

Ethanol
Large concentrations of ethanol are toxic to yeast, reason why there is an inhibitory fraction in the production rate of the biomass. In the Equations 2.55, 2.56 and 2.57 is clear that if ethanol is equal to zero, the terms where E appears ($e^{-k_{iE}E}$) will be equal to one, meaning that if there is no ethanol on the wort, there is no inhibition fraction in the growth rate, this also means that this equations are false and the observability matrix rank will be 3. For the case where there is too much ethanol the term will go to zero so all the equations will be true and the rank will be 2. Very different from the case where there is only CTR measurement where ethanol concentration does not even appear in the Equation 2.60. Which means that it does not affect the observability matrix rank.

Conclusion
In normal parameters, the system will be locally observable independent on its state variables for the case where it is measured the carbon dioxide transfer rate from liquid to gas and the substrate or only the carbon dioxide transfer rate from liquid to gas. If there is a highly concentration of ethanol or a no-existence of substrate the system becomes unobservable, for the first situation, but this cases are considered out of the scope since they inhibit the reaction.
A good remark is that the observability along the trajectory gives an indication about global observability by checking it locally in several points. Thus a true observability is not demonstrated. Therefore a practical observability may differ from local observability.

Summary

Configuration	**Local Observability Condition**
CTR and S measured	$S \neq 0$ $X \neq 0$ E too big tending to infinite
Only CTR measured	$S \neq 0$ $X \neq 0$

Table 2.1: Summary of Local Observability Analysis of Proposed Model

Chapter 3

Prediction of States

In the fermentation process, there are some variables that can be modelled: biomass, extract, ethanol and by-products concentrations, CO_2, oxygen, and other gasses emission, temperature, pressure, specific gravity, pH, chemical composition, spectral composition etc. Nowadays there are technologies to measure all of these variables and much more, but most of them can only be measured off-line. Because of that, this project proposes to implement an observer to predict some of these process variables.
In control theory, an observer is a system that provides estimate of internal states of a system. So, if one could measure one or more states, with an observer it might be possible to estimate the rest of the state variables.
In Chapter 2.3 it was proven that if CTR and the substrate was measured the system is observable, so it means that with these signals, it is possible to create an observer that can estimate the rest of the state variables (ethanol and biomass).

3.1 Extended Kalman Filter

The Kalman Filter (KF) is a stochastic observer or recursive estimator which is considered as optimal in the sense of the variance, under specific noise distribution assumption (Gaussian white noise distribution) [32]. Since the KF is limited to linear systems, an extended version to non-linear systems has been proposed and denoted Extended Kalman Filter (EKF).
This type of observer has different versions. The one used in this project is the continuous-discrete EKF where the model is in continuous time and the measurements is in discrete. This type of observer is used mainly for a non-linear system like 3.1, where $x(t)$ is the state variable, $y(t_k)$ is the measurements, t is the continuous time and t_k is the discrete time.

$$\begin{cases} \dot{\underline{x}}(t) = \underline{f}(\underline{x}(t), \underline{u}(t)) + \underline{w}(t) \\ \underline{y}(t_k) = h(\underline{x}(t_k)) + \underline{v}(t_k) \end{cases} \tag{3.1}$$

Also $\underline{w}(t) \sim \mathcal{N}(0, Q(t))$, $\underline{v}(t_k) \sim \mathcal{N}(0, R)$, $\underline{f}(\underline{x}(t), \underline{u}(t))$ and $h(\underline{x}(t_k))$ are non-linear functions and $\underline{w}(t)$ and $\underline{v}(t_k)$ are respectively the model and measurement noises.
The EKF algorithm consists in two main parts: the prediction and the correction. Figure 3.1 proposes a graphical representation of the sequence of the observer. At the beginning, the system takes the initial state estimations and the initial covariance matrix P_0 in consideration and at each sampling time the observer will pass through first the prediction than the correction part. And since this EKF is a continuous/Discrete Kalman Filter the prediction part is in continuous while the correction part is in discrete.
The prediction starts by propagating the state variable estimates from the previous sampling time to the new one, as seen in Figure 3.1. After, it is necessary to calculate Jacobian of the state transition function $\underline{f}(\underline{x}(t),\underline{u}(t))$, denoted A, to proceed to the prediction by calculating

the Predicted Covariance Matrix (P). The correction starts by calculating the output matrix C. The observer Gain is calculated using the predicted covariance matrix and the output matrix. With the gain calculated it is possible to predict the new values for the state variables and the covariance matrix of the next timestep. After this steps, the system will go to the next timestep and there is a restart of the calculations with the new values just calculated for the covariance matrix and state variables.

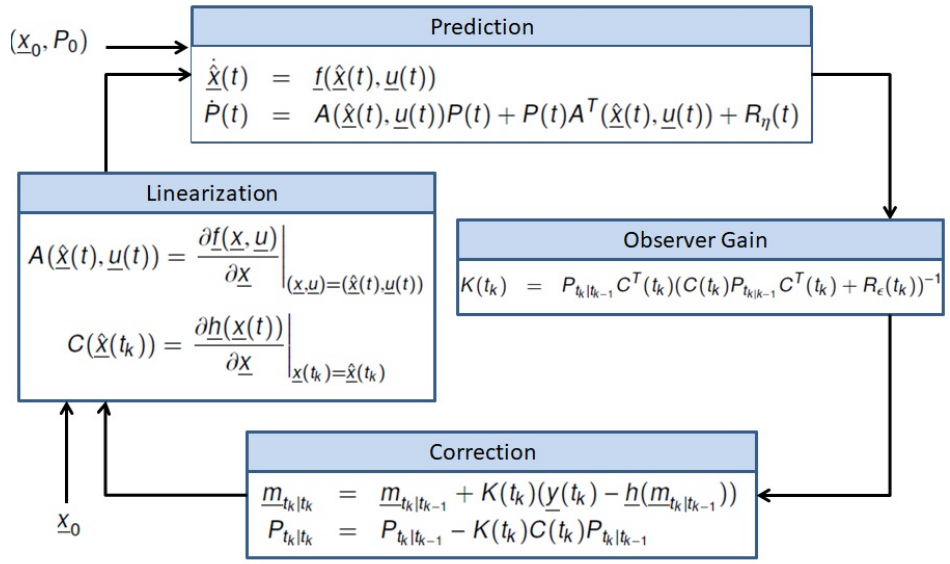


Figure 3.1: Extended Kalman filter Scheme

The extended kalman filter uses a linear approximation of a non-linear system. This approach is therefore not optimal. Also, if there is a model error or a bad initial state estimation, the EKF may diverge due to the linearisation. Another disadvantage is the fact that the approximation may not be the best possible one so it leads to a bad performance of the EKF due to the fact that the approximation is based on a single point per iteration which may cause statistical inconsistency.

3.2 Analytical Calculation of the EKF

The system (3.2) which the EKF will be applied is the novel model, from Subsection 2.2, where the state variables are S, E and X, the initial conditions are $S(t_0) = S_0$, $X(t_0) = X_0$, $E(t_0) = E_0$ and the measurement is given by $CTR = k_{CTR}\ \mu_X X$ and S.

$$\begin{cases} \dot{S} = -k_{SX}\ \mu_X\ X \\ \dot{X} = \mu_X\ X \\ \dot{E} = k_{EX}\ \mu_X\ X \end{cases} \quad , \ \mu_X = \mu_{max} \frac{S}{K_X + S}\ e^{-k_{iE}\ E} \tag{3.2}$$

Prediction
The prediction model is:

$$\dot{\hat{S}}(t) = -k_{SX}\ \mu_{max} \frac{\hat{S}(t)}{K_X + \hat{S}(t)}\ e^{-k_{iE}\ \hat{E}(t)} \hat{X}(t) \tag{3.3}$$

$$\dot{\hat{E}}(t) = k_{EX}\ \mu_{max} \frac{\hat{S}(t)}{K_X + \hat{S}(t)}\ e^{-k_{iE}\ \hat{E}(t)} \hat{X}(t) \tag{3.4}$$

$$\dot{\hat{X}}(t) = \mu_{max}\frac{\hat{S}(t)}{K_X + \hat{S}(t)}\ e^{-k_{iE}\ \hat{E}(t)}\hat{X}(t) \tag{3.5}$$

The predicted covariance matrix P is given by $\dot{P}(t) = A(\underline{\hat{x}}(t))P(t) + P(t)A^T(\underline{\hat{x}}(t)) + Q(t)$ where the state transition matrix A_k is a Jacobian and $Q(t)$ is the process noise covariance matrix that describes the system noise.

$$A = \left.\frac{\partial f}{\partial x}\right|_{\underline{\hat{x}}(t)} \tag{3.6}$$

$$A = \begin{bmatrix} \frac{\partial \dot{\hat{S}}(t)}{\partial \hat{S}(t)} & \frac{\partial \dot{\hat{S}}(t)}{\partial \hat{E}(t)} & \frac{\partial \dot{\hat{S}}(t)}{\partial \hat{X}(t)} \\ \frac{\partial \dot{\hat{X}}(t)}{\partial \hat{S}(t)} & \frac{\partial \dot{\hat{X}}(t)}{\partial \hat{E}(t)} & \frac{\partial \dot{\hat{X}}(t)}{\partial \hat{X}(t)} \\ \frac{\partial \dot{\hat{E}}(t)}{\partial \hat{S}(t)} & \frac{\partial \dot{\hat{E}}(t)}{\partial \hat{E}(t)} & \frac{\partial \dot{\hat{E}}(t)}{\partial \hat{X}(t)} \end{bmatrix} \tag{3.7}$$

$$A = \begin{bmatrix} -\frac{k_{SX}\ \mu_{max}K_X e^{-k_{iE}\ \hat{E}}\ \hat{X}}{(K_X+\hat{S})^2} & \frac{k_{SX}\mu_{max}\ k_{iE}\ e^{-k_{iE}\ \hat{E}}\ \hat{X}\ \hat{S}}{K_X+\hat{S}} & -\frac{k_{SX}\mu_{max}\ e^{-k_{iE}\ \hat{E}}\ \hat{S}}{K_X+\hat{S}} \\ \frac{k_{EX}\mu_{max}K_X e^{-k_{iE}\ \hat{E}}\ \hat{X}}{(K_X+\hat{S})^2} & -\frac{k_{EX}\mu_{max}\ k_{iE}\ e^{-k_{iE}\ \hat{E}}\ \hat{X}\ \hat{S}}{K_X+\hat{S}} & \frac{k_{EX}\mu_{max}\ e^{-k_{iE}\ \hat{E}}\ \hat{S}}{K_X+\hat{S}} \\ \frac{\mu_{max}K_X e^{-k_{iE}\ \hat{E}}\ \hat{X}}{(K_X+\hat{S})^2} & -\frac{\mu_{max}\ k_{iE}\ e^{-k_{iE}\ \hat{E}}\ \hat{S}\ \hat{X}}{K_X+\hat{S}} & \frac{\mu_{max}\ \hat{S}\ e^{-k_{iE}\ \hat{E}}}{K_X+\hat{S}} \end{bmatrix} \tag{3.8}$$

$$\dot{P}(t) = AP + PA^T + Q \tag{3.9}$$

The covariance matrix $Q(t)$ will be designed considering the expected level of uncertainty on the model and prevents the correction matrix $K(t)$ from disappearing when $t \to \infty$. If this matrix is set to zero, it means that the model matches perfectly the real system, which is not the case.

Correction

The correction part is a discrete system, different from the prediction part which is continuous. The observer gain is calculated using the output matrix C calculated along the state trajectory.

$$C = \left.\frac{\partial h}{\partial x}\right|_{\underline{\hat{x}}(t)} = \begin{bmatrix} \frac{\partial C\hat{T}R(t)}{\partial \hat{S}(t)} & \frac{\partial C\hat{T}R(t)}{\partial \hat{E}(t)} & \frac{\partial C\hat{T}R(t)}{\partial \hat{X}(t)} \\ 1 & 0 & 0 \end{bmatrix} \tag{3.10}$$

$$C = \begin{bmatrix} \frac{k_{CTR}\ \mu_{max}K_X e^{-k_{iE}\ \hat{E}}\ \hat{X}}{(K_X+\hat{S})^2} & -\frac{k_{CTR}\mu_{max}\ k_{iE}\ e^{-k_{iE}\ \hat{E}}\ \hat{X}\ \hat{S}}{K_X+\hat{S}} & \frac{k_{CTR}\mu_{max}\ e^{-k_{iE}\ \hat{E}}\ \hat{S}}{K_X+\hat{S}} \\ 1 & 0 & 0 \end{bmatrix} \tag{3.11}$$

So the observer gain (3.12) will be given by Equation 3.12.

$$K(t_k) = P_{t_k|t_{k-1}}C^T(t_k)\left[C(t_k)P_{t_k|t_{k-1}}C^T(t_k) + R\right]^{-1} \tag{3.12}$$

And the updates of the state estimate and covariance estimate will be Equations 3.13 and 3.14 respectively.

$$\hat{x}_{t_k|t_k} = \hat{x}_{t_k|t_{k-1}} + K(t_k)[y(t_k) - h(\hat{x}_{t_k|t_{k-1}})] \tag{3.13}$$

$$P_{t_k|t_k} = P_{t_k|t_{k-1}} - K(t_k)C(t_k)P_{t_k|t_{k-1}} \tag{3.14}$$

3.3 Simulation of the EKF

For the execution of the model, the parameter values were taken from the Reference [10], that can be seen in Annexe II. It was chosen parameter values for Saccharomyces cerevisiae S-33 (top fermented yeasts) for Immobilized cells with Aiba kinetics with 13% OE and the translation to this project's model can be seen in Appendix B in Section B.3.
The implementation of the model and the observer is in MATLAB® Script. The model is implemented using a non-stiff differential equation solver (ode45 [23]) while the observer follows the method explained in Section 3.1 and uses the analytical calculations of the Section 3.2.
For a realistic simulation, the chosen process time was 20 days with a sampling time of 10 minute. These values were chosen because the primary fermentation process for beer takes from two to three weeks in general and in the peak of the fermentation, this sampling time will be needed to capture the emitted bubbles when the measurement system is coupled.
The initial state variables for the model are taken from the reference [10], and the initial state estimation for biomass and substrate are a random value in a possible range. The initial state estimation of ethanol is zero because there is the assurance of the absence of this concentration at the beginning of the fermentation process.
It is considered that the system measures CTR and specific gravity so the measurements were calculated using the model output and noise elements were added of an order of 5% of their signal to give a realistic behaviour.
The initial covariance matrix P_0 (3.15) should reflect the confidence in the initial state estimates. If the initial state estimation is trust worthy the initial covariance value is small, but if it is the opposite and the initial state estimation is unreliable, the initial covariance value is big. Since the biomass and the substrate initial state estimations are random, their initial covariance value will be big and for ethanol the initial covariance will be very small because of the assurance of the initial value.

$$P_0 = \begin{bmatrix} 0.04 & 0 & 0 \\ 0 & 0.00001 & 0 \\ 0 & 0 & 0.04 \end{bmatrix} \tag{3.15}$$

The measurement noise covariance matrix R will be given by 3.16 and it is matrix representing a noise of 5% of the order of the signals.

$$R = \begin{bmatrix} 0.0025 & 0 \\ 0 & 0.25 \end{bmatrix} \tag{3.16}$$

The process noise covariance matrix Q (3.17) should be chosen in accordance with the level of confidence in the model. Good practice suggests to assess if the measurements are more trustworthy than the model or not. If yes, Q should be smaller than R, if not, Q maybe be chosen at the same order of magnitude or greater than R. It needs to maintain the uncertainty to keep the gain in steady state working, as explained at the sub section 3.2.

$$Q = \begin{bmatrix} 0.04 & 0 & 0 \\ 0 & 0.0025 & 0 \\ 0 & 0 & 0.00025 \end{bmatrix} \tag{3.17}$$

The diagonal of the covariance matrix P represents the square of the variances of the state estimation errors. If this standard deviation is multiplied by two, it gives a 95% confidence interval for the value estimated. If these values is big, means that the deviation is also big and the uncertainty of the value is greater, with the opposite being reciprocal. So the confidence interval is calculated to estimate how trustworthy the estimations are.

It is important to check two things: The convergence of the observed state at the beginning, approximating it to the values to the real system; The possible loss of the observability along the trajectory, checking if the observed variables continue to follow their real counterparts.

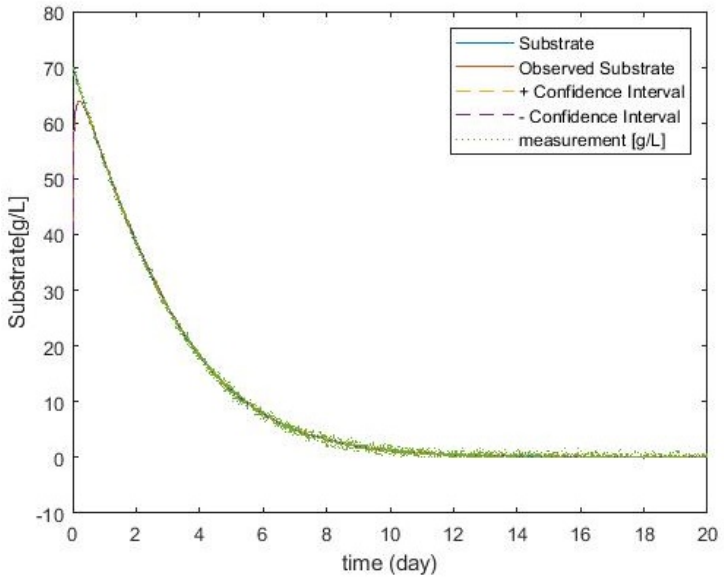
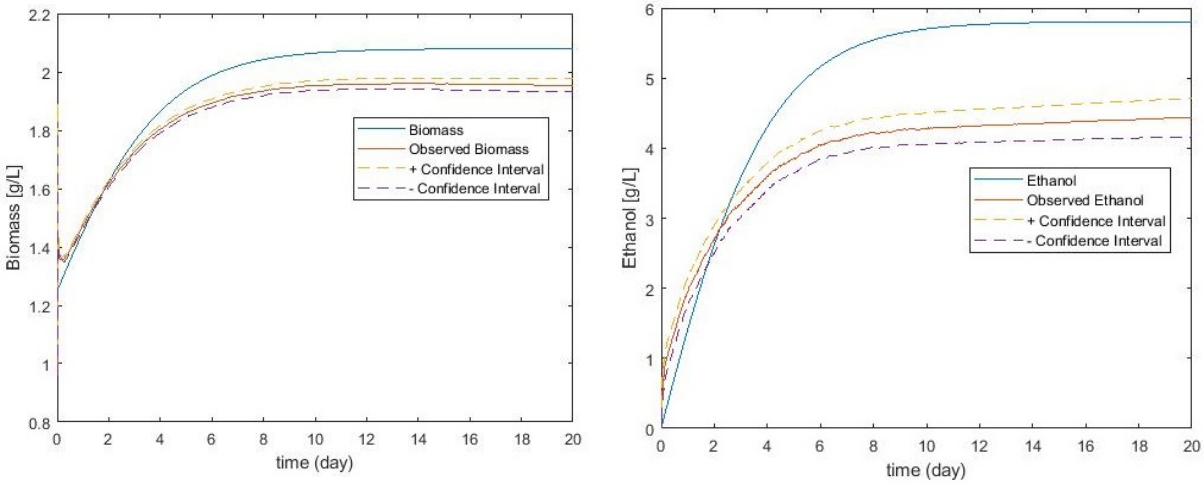


Figure 3.2: Substrate consumption

The substrate, is one of the measured variables, so the observed state variable relative to the substrate concentration should be equal to the original state variable. The observed substrate takes almost a day to follow the right trajectory, as seen in Figure 3.2 and it maintains a good path until the end, as expected.
The observed biomass and ethanol are non-measured variables and both do not present an optimal behaviour. For the biomass it has a good convergence at the beginning, but it looses observability along the trajectory after the second day and for the ethanol, there is no apparent convergence in the beginning and it also looses observability along the trajectory after the second day. The loss of observability and the convergence error of the ethanol should be checked.



(a) (b)

Figure 3.3: Simulation of: (a) Biomass production; (b) Ethanol production.

The loss of observability can be cause by many factors, one of them is the non-linearity characteristics of the system combined with the linearization of the EKF causing an already explained, differ between practical observability and local observability. Which means that the measured variables are probably reaching a boundary condition and with that, loosing the observability of the system. So tests about the measured variables need to be done. And more details about this is discussed in Chapter 5.
So the CTR evolution (Figure 3.4) is an indicative of the lost of observability. One thing to observe is its order. It is a very small sign compare to the magnitude of the rest of the parameter values, after the forth day it starts to get values closer to zero, that can lead to an approximation to zero. Another interesting observation is that this measurement totally vanishes after two weeks. Because of this elements, a dilution rate was tested to extend the signal throughout the 20 days of simulation and apparently the local trajectory observability got better.

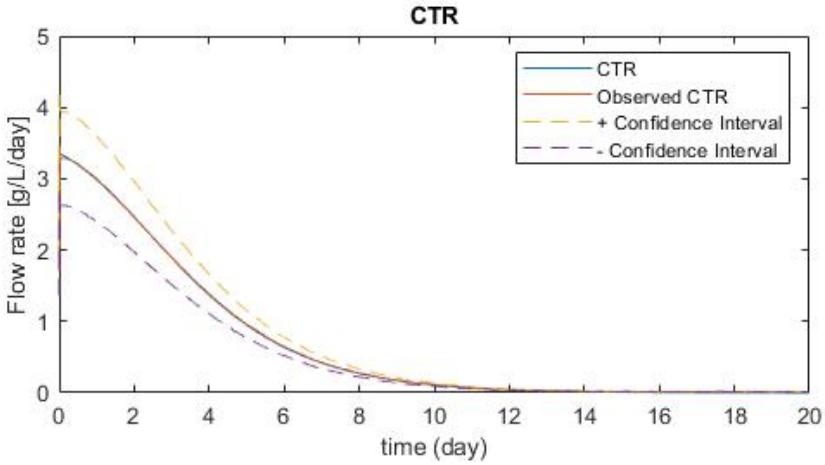


Figure 3.4: Biomass production

Chapter 4

Experimental Studies

4.1 Data Acquisition

4.1.1 Specific Gravity acquisition system

This project had two sensors for measuring specific gravity, a refractometer and an hydrometer. The digital refractometer was used to sample the specific gravity throughout the process, specially the mashing, where the hydrometer couldn't actuate. This device is an off-line sensor that needs a sample of the wort/beer to process the value, so it creates troubles. An example of issues encountered is when opening the valve to take the wort/beer. It makes a drop of the pressure, making the solution, that is inside of the airlock, go inside of the fermenter.

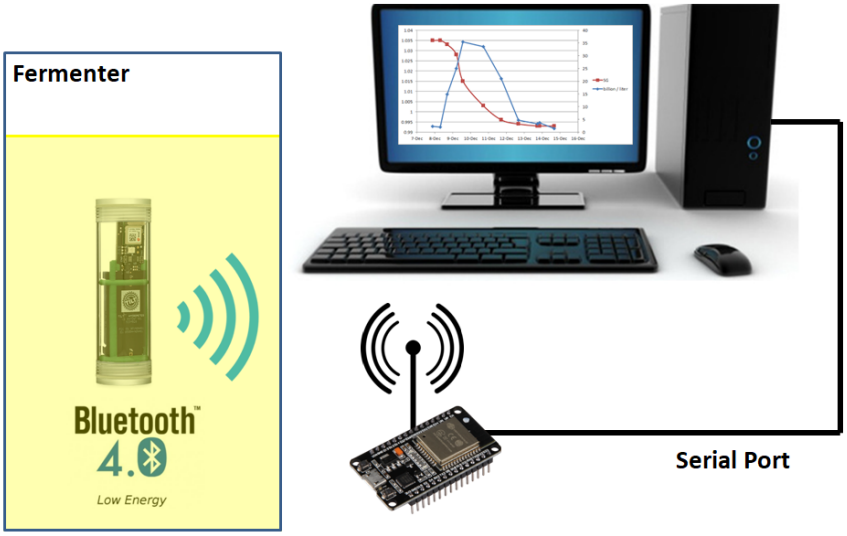


Figure 4.1: Specific Gravity Acquisition System Set-up

The hydrometer (more information about it can be seen in Appendix D in Subsection D.2.2.2) is perfect for homebrewers, but it is not ideal for this project due to the fact that for acquiring the signal one must be close to the device, with an smartphone with the Tilt App and press a buttom every time one wants to send the data (measurements plus time) to the cloud, meaning that this device does not save its measurements on its own.
To save the measurements an acquisition system composed by the sensor, a microcontroller and a computer was developed as seen in Figure 4.1. Resuming the data path, the sensor measures the wort/beer than sends a message via Bluetooth® to the microcontroller that will translate it and send this data to the computer that will save it in an excel file.
Tilt™ sends raw iBeacon messages by Bluetooth®, so the microcontroller needs to receive this messages, unpack, translate, select them than send only the data needed to the computer through

the serial port. The data format that Tilt™ transmits and the unwrapping of the data for transmission can be seen in Appendix D in Subsection D.2.2.2.
The microcontroller used in this project was the ESP32 which is a low-power microcontroller integrated with Wi-Fi and dual-mode Bluetooth®. It was chosen because it can be programmed in ARDUINO® Integrated Development Environment (IDE) and its wireless connectivity is compatible with the sensor, Bluetooth® v 4.0+.

4.1.2 Released CO_2 rate acquisition system

The released CO_2 rate (CTR) measurement is one of the bases for this project. To measure CTR it was created an acquisition system and its sensor.
For the acquisition system three types of microcontrollers were tested: ARDUINO®, ESP8266 and ESP32. They were chosen due to their availability, the developer familiarity, all could be programmed in ARDUINO® IDE and they appeared to fit the design requirements. ARDUINO® and ESP8266 were discarded from the project with time. ESP8266 biggest feature is his Wi-fi connection, which at the beginning was needed but due to changes of the project's sensor, this feature was no longer needed, so ESP8266 was not implemented. ARDUINO® was implemented in two of the versions of the sensors and it presented an overflow of the memory loosing part of the measurement, so it was discarded due to lack of trust. So, ESP32 was the microcontroller chosen by elimination.
To create the sensor, the idea is if one measures the quantity of bubbles emitted in the airlock per a fraction of time, it is measuring a rate, and this can be related to the CTR. So, if the airlock has a physical movement (1 or 2 in Figure D.1), it is possible to couple a sensor to it and calculate this movement. The movement of the airlock is described in Appendix D in Paragraph §2.

4.1.2.1 First Version: Simple hall sensor with one big magnet

For sensoring these points, the first version uses a simple hall effect sensor coupled in the structure with a magnet coupled in the moving part. This type of sensor gives binary values, following the Figure 4.2, if it sensors magnetic field it switches on, if it goes out of the magnetic field, it switches back off. So, it is possible to calculate the emitted bubbles setting a trash hold.

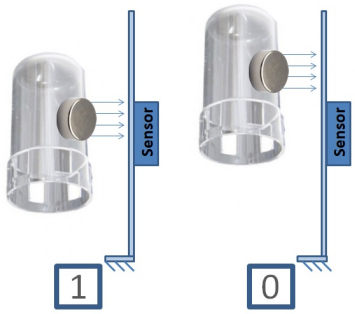


Figure 4.2: Version one sensor mechanism

The first sensor was created with the resources available in the laboratory. Due to this fact, this version failed in the first brewing it was implemented and it had to be upgraded. During the experimental process, the moving part of the airlock rotated making the measurement impossible, since the sensor is fixed into the structure. Also, the magnet that was available is weak, associated with a simple hall effect sensor generated a big loss of accuracy.

4.1.2.2 Second Version: Simple hall sensor with a ring of magnets

Figure 4.3: CO_2 Sensor Version 2

Created to upgrade the first version and fix its problems, the second version of the CO_2 was the most studied and implemented version. Implemented with the same simple hall effect sensor, the highlight of this version is the manipulation of the magnetic field and the implementation of strong magnets.
To fix the rotation problem of the airlock, the idea was to make a magnetic ring that does not depend on the rotation of the moving part. So, it was created a ring of magnets in the moving part, as seen in Figure 4.3. So the system now has a stronger magnetic field, all around the airlock, that the sensor can easily sense.
During experimental tests it was verified a need to change the type of sensor. The switching characteristic of the sensor and its sensitivity associated with corrosion and a natural vertical displacement of the moving part resting point of the airlock caused by pressure created general loss of data. Due to these issues, lapses of time with no measurements were occurring even thought the movement and the fermentation were still happening, and this is unacceptable.

4.1.2.3 Third Version: Ultrasonic sensor

The third version uses the Speidel airlock (number 3 in D.1) and an HC-SR04 ultrasonic sensor for ARDUINO®. Following the Figure 4.4, it determines the distance of the airlock top with respect to the sensor that is coupled to an external platform. To capture the emitted bubbles, a posteriori data treatment needs to be done.

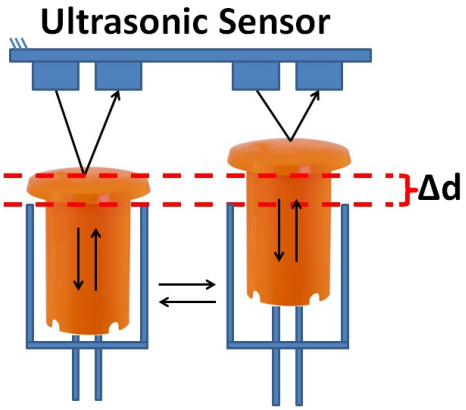


Figure 4.4: Mechanism of Ultrasonic Detection

The advantage of this version is the fact that the sensor has no contact with the fermenter. If there is an over fermentation and their is an overflow of foam, there won't be a risk to the acquisition system. Also it is easier to sanitize and maintain this version since there is no magnetic neither electronic component attached to the airlock.
This version was tested in a batch of 20L, which was the only fermenter the laboratory had that fit this versions spout, and it failed. The reason why is that this sensor has a resolution of 0.3 cm and the blop makes a movement that can reach from 0.1 to 0.4 cm depending on its intensity. When the fermentation is in its peak, the majority of the emitted bubbles movements are smaller but frequent so the sensor stops sensing the different distance.

4.1.2.4 Final Version: Linear hall sensor with a ring of magnets

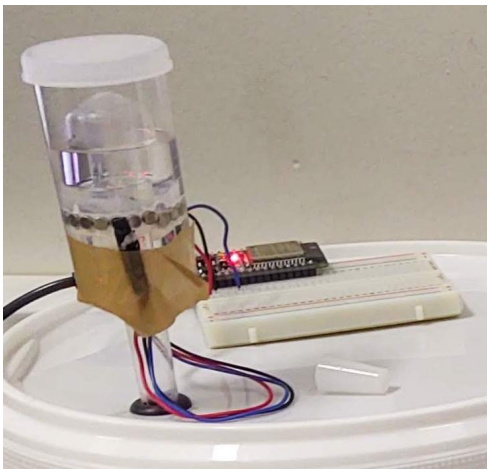


Figure 4.5: CO_2 Sensor Version 4

The final version changed the type of hall effect sensor to a linear one but it maintained the magnetic ring. The linear hall sensor, different from the previous version sensor that gave binary values, measures the power of the magnetic field.
Like the third version, it also needs a posteriori data treatment to count the emitted bubbles. Also, this measurement is directly related to the movement of the airlock, so figures like D.3 could be visualised.
Since it gives the position of the airlocks moving part during time, this version also provides a solution for the error that came from the displacement caused by pressure, which is one of the main issues of versions 2 and 3.

4.2 Set-up

Figure 4.6: Set-Up of Fermentation process

To brew a beer one needs very few equipments, but to make a good one and replicate it, one needs a wide range of equipments, such as: cooking pots, sensors, cables, buckets, chillers, pipes, fermenter, pots, thermometer, hydrometer, diverse types of kitchen utensils, refrigerator, grainer, filters, controllers, among others. These equipments can be found in different shapes from the cheapest to the most expensive. A review for the equipments used in this project can be found in Appendix D in Section D.2.
The ingredients and the recipes came from the same provider in brewing kits for 20L batch. All recipes were chosen to have a short period of fermentation and with similar styles of beer. Due to the lack of temperature control in the fermentation process, it was chosen to produce only ales that ferments in temperatures around 20 to 22^{o}C.
Two experimental set-ups were employed for this project, one for the wort production and one for the fermentation experiments.
The wort production, that consists in the processes of milling, mashing and boiling, has its set-up consisting in the Grainfather G30 brewing kit and a miller. This set-up supports a production of 30L batches but the experiments were done for 20L batches per day per experiment. It lasts a day and all brewing processes explained in section 1.2 until the fermentation are done in this frame of time. Also, it starts and ends with cleaning and sanitization of the equipment, this step is primordial for the maintenance of the equipment and the success of the fermentation process.
In the fermentation process to amplify the quantity of data acquisition and to optimize the work done and reduce time in the laboratory the 20L batch of wort production was divided in batches of two or three. In the batches of two, it was used carboy PET fermenters of 12L and in the batches of three it was used food buckets of 6L. Both set-ups have the same structure and the only configuration that changes is the acquisition system.
The fermentation set-up, Figure 4.6, is an open loop system, with no temperature control. It was used in each experiment a set of 2 or 3 fermenters, depending on the availability and the quantity of wort, filled up to 75% of its capacity with wort and inoculated with a yeast suspension.
Experiments were replicated seven times to acquire experimental data. This project was assisted by already known instrumentation devices but it was also created two acquisition systems, one for the specific gravity and the other for CO_2 released for the fermenter. A review of the instrumentation devices can be seen in Appendix D in Section D.2.2.
Specific gravity was measured off-line at the beginning and once a day until the end of the fermentation. There was the attempt of measuring the specific gravity on-line, and an acquisition system for that propose was created, which was a requirement of this project, but it failed.

Released CO_2 rate was measured on-line by the sensors created in this project. The data passes through the microcontroller via a wired connection. Than, the microcontroller sends this data to a computer, also via a wired connection, that saves the measurements in an excel file. The softwares used to send the data from the sensor till the final end, and more details about this process, can be seen in Appendix D in Section D.2.3.

4.3 Data Treatment

It was implemented two different data treatments for the measurement coming from the acquisition system of CTR due to the different versions of the sensors.
For the first CTR sensors, where it was implemented the simple hall effect sensoring (first and second version), the emitted bubbles coming from the airlock where counted, so the rate was based on the number of bubbles. The data simply needed to passed through a low pass filter and a fitting process to be proper to use as shown in Figure 4.7.

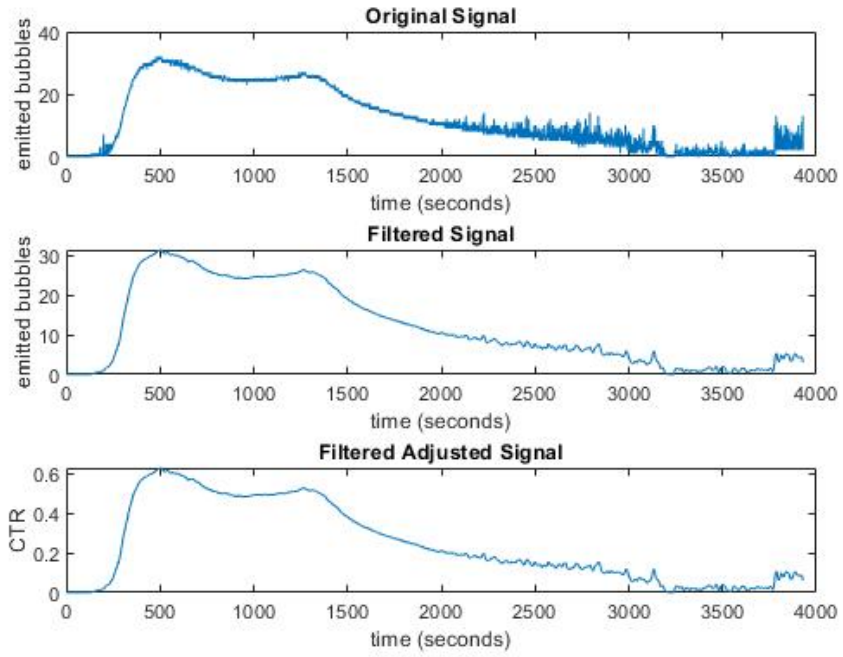


Figure 4.7: Data Treatment process for the first type of sensors of CTR

The final CTR sensor has a different hall effect sensor and because of this configuration, the data treatment is more complex. One must count the emitted bubbles, than make the CTR signal. For counting the bubbles, it was used a function for finding peaks created by [33] and after finding them, they were joined in fractions of time to form the rate. To finish, it was adjusted the value gain to the CTR gain. Figure 4.8 shows the step-by-step of the method applied.

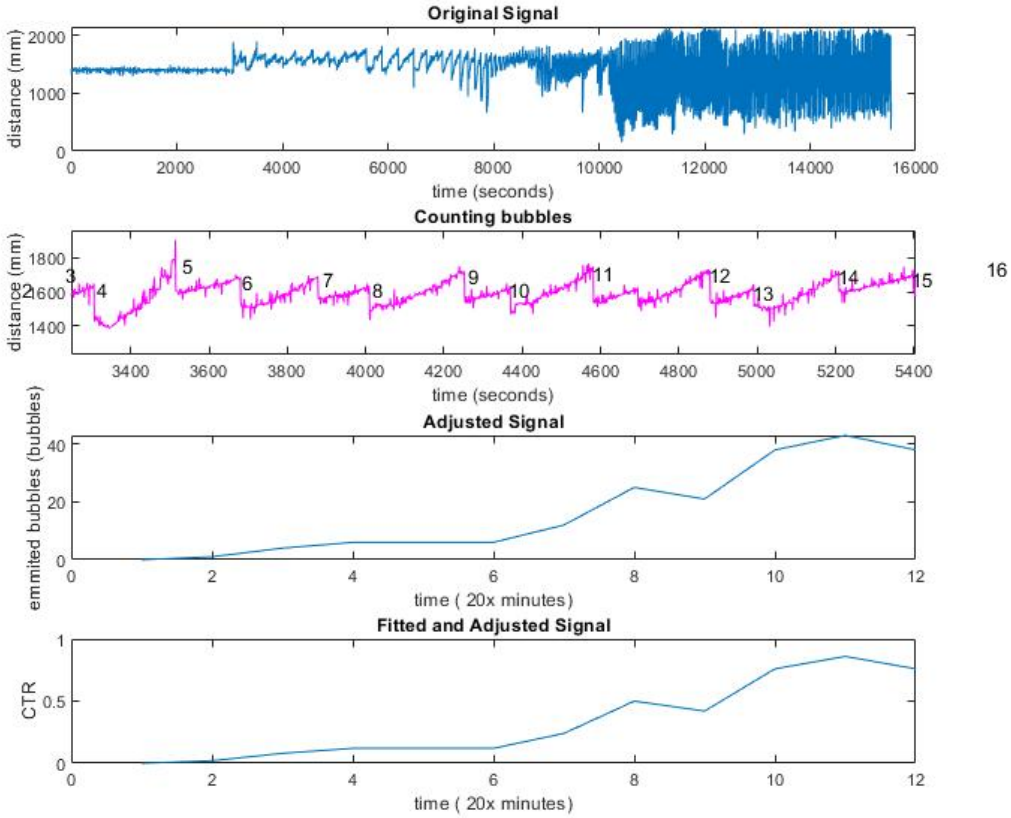


Figure 4.8: Data Treatment process for the final type of sensors of CTR

4.4 Kalman Filter Test

Since none of the experimental data can be used to test the observer due to the encountered difficulties, this experimental test will be done with simulated models. These models are selected from a bibliographic review.

The extended kalman filter proved to be robust to strong measurement noise (Figure 4.9) but lacked precision when coupled with different models, specially for the biomass signal. The increasing of the lack of precision, leading to a bad estimation, associated with the aspects of the extended Kalman filter designed, makes the system loose its observability with time.

The confidence interval changed for all the tests, specially for the ethanol and biomass signals. For the ethanol, there was an increase in the tilt angle of the confidence interval. The biomass signal, depends a lot on the initial condition and noise, the confidence interval sometimes would get a similar shape as the substrate and the biomass signal would loose observability only at the end of the process, but in other cases it would have a ramp shape with a high tilt angle and with a lost of observability at the beginning.

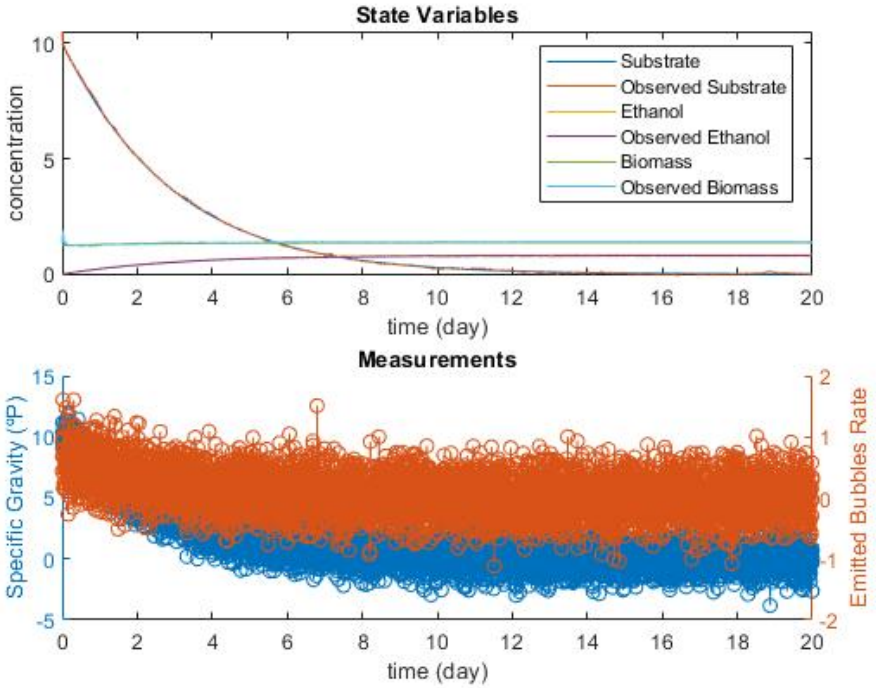


Figure 4.9: All variables together

4.5 Encountered Difficulties and Solutions

During the experimental phase of the project, problems in the acquisition system and with the biological process happened. This section is dedicated to discuss about this encountered difficulties and its solutions.

4.5.1 Acquisition System Problems

One of the main issues encountered was the time constraints versus equipment availability. The number of sensors designed, quantity of microcontrollers and fermenters should be directly affected by time constraints such as fermenting time range and project due time. But, for this project, this points were more affected by the availability in the laboratory and providers than the time constraints.
There were some problems encountered with the microcontrolers, one of them was a memory RAM overflow. Some of the fermentation processes went smoothly, and the sensors seemed working but when the data was checked figures like 4.10 were plotted. It was discovered that ARDUINO® UNO has a RAM memory of 32K bytes, that was saving the data transmitted in the serial port and couldn't support the quantity of data flow of the system while the ESP32 has 520K bytes and did not save this data. So the solution encountered was to exclusively use ESP32 as the microcontroler.

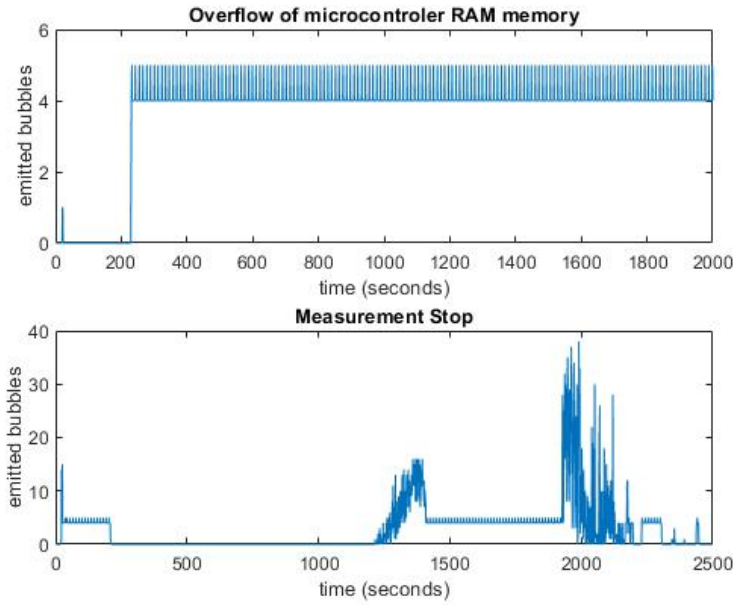


Figure 4.10: Acquisition errors. 1st: Overflow of microcontroler RAM memory. 2nd: Measurement Stop

Due to the lack of the microcontroler availability, there was a need to unify the acquisition system for both measurement. Use two sensors, same microcontroler. The unification failed, so one had to prioritize one of the on-line measurements. It was chosen to discard the on-line measurement of specific gravity and maintain an off-line measurement since the released CO_2 rate measurement could only operate with the microcontroler in an on-line system.
Another common problem occurring with the experiments was the stop of the measurement due to external facts. Energy instability, connection instability and computer shut-downs during the middle of the fermentation. Most of these issues were solved as soon as discovered, but a lot of experiments were lost due to these problems because they could happen in the middle of the night where there was no one to fix.
The airlock design and implementation also had problems. Some problems and its solutions were already explained in the sub section 4.1.2, but the biggest among the airlock problems is the corrosion of the magnets.

Figure 4.11: First version of CTR Sensor

The first experiments were carried out with airlocks half filled with water as specified. Water showed to be very corrosive towards the magnet of the first version, as seen in Figure 4.11, one can

clearly see the line that separates the portion of the magnet in contact with water and without it. Water also prove to be corrosive, but in small portion, towards the strong magnets and the glue, destroying sensors like in Figure 4.12.

Figure 4.12: Destroyed CO_2 sensor

The replacement of water for alcohol 70% in the airlocks came to try to fix the corrosion and the contamination problems. Alcohol 70% is less corrosive than water, but it did not prevent the corrosion to happen, just considerably slowed.
An example of situations that happened with corrosion is the case of Figure 4.13. This airlock moving part locked due to the magnets that escaped from the magnetic ring and joined with their neighbour magnets. This locked airlock created a over pressure in the fermenter overflowing the wort and biomass that was inside. The orange liquid of the figure is a mix of alcohol and wort and the beige liquid is biomass.

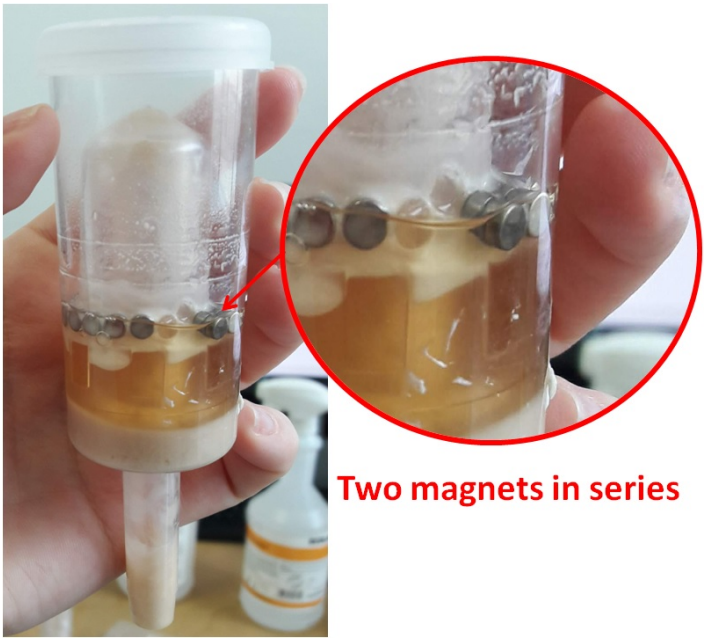


Figure 4.13: Destroyed CO_2 sensor with locked moving part

A cheap solution encountered was to apply a coat of nail polish to form a protective layer, as seen in Figure 4.14. And since this solution is not an optimal solution, the nail polish must be passed in the sensor before every use, and that is why it is possible to see layers in Figure 4.14.

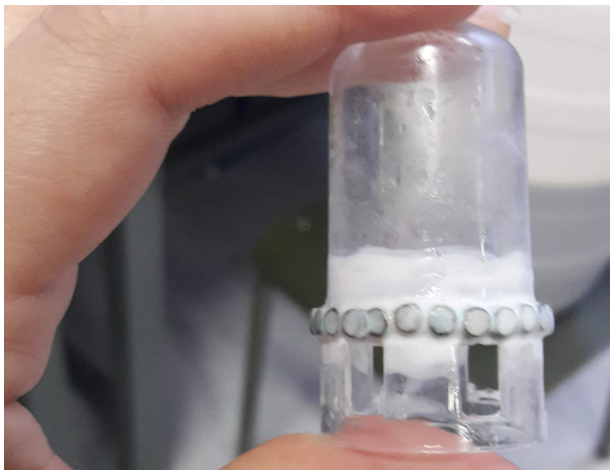


Figure 4.14: Moving part of Airlock with magnetic ring and protective layer after an experiment

4.5.2 Brewing Problems

Because brewing is a biological process and have a lot of nuances and steps to follow, problems occur all the time. In the literature it is possible to find guides to help Brewers recognise and solve this problems. J. J. Palmer, in his book, *How to brew* [1], dedicates a chapter to discuss this matter. This chapter was used as a guide for this project.
From the first experiment on, the project suffered constantly with ruined beer. Beer that were not good for consumption due to contamination or strong off-flavours. This issues not only ruin the consumption of the production but also the quality of the fermentation process, so the next sub sections are dedicated to discuss them.

4.5.2.1 Contamination

The contamination most of the time happens when the wort is exposed to the air, so in between processes is when there is a high chance of contamination. Between boiling and the primary fermentation, primary fermentation and priming, priming and bottling are critic times where contamination could occur in this project, where the first one would affect directly the measurements.
Figure 4.15 represents a case where a contamination affected directly the measurement. In this case, there was a contamination of bacteria on the primary fermentation. The curve of emitted bubbles over times shows two peaks presenting a possible second specie in the wort fermenting the sugar as well as the yeast. A further investigation needed to be done to prove if this suspicion confirms.
If, after the primary fermentation, no smell is present, meaning that the fermentation is complete without problems, all the production passes through priming and are bottled for the second fermentation. And After two weeks of prime, the beer are ready for consumption if it was not contaminated.
Before consumption, the beer would pass through a final visual, taste and smell test to determine if it was appropriate for consumption and most of the production would fail this test. This test would consist in open a bottle in room temperature, serve it in a glass, check the foam, gas, smell and flavour of the liquid.
The characteristics that accused the contamination by bacteria were the acidic taste and the vinegar smell. Following [34] there has some bacteria that can spoilage beer like lactic acid bacteria, acetic acid, Enterobacteriacea, Pectinatus, Megasphaera and Zymomonas and since it was impossible to do a laboratory test to determine what species of bacteria was, the beer was disposed.

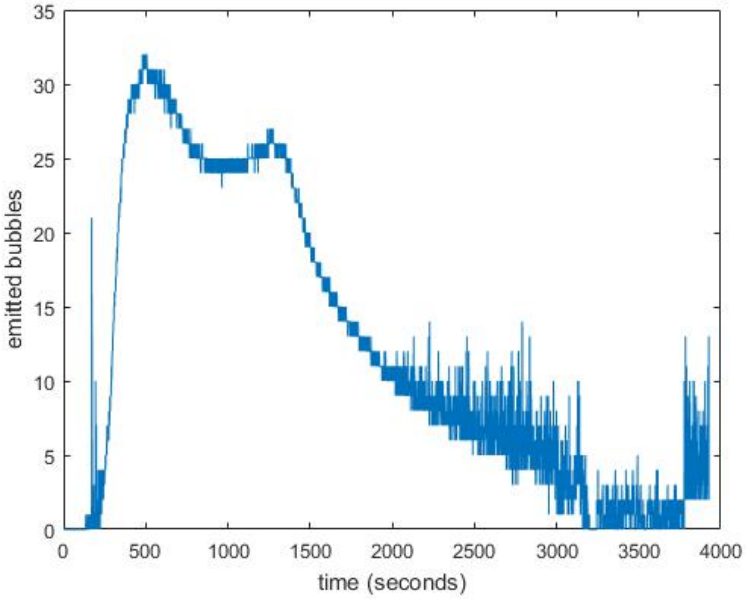


Figure 4.15: Chart of bubbles emission over time of contaminated wort

J. J. Palmer says in [1] that for smells like vinegar *Aceto bacteria* and *Lacto bacteria* are the most common cases in breweries. And this bacteria produces characteristics that are appreciated for some styles of beer that if the Brewer desires the flavours, it could keep it since it is harmless.

4.5.2.2 Yeast

Yeast is a living been. And as a living been, it has behaviour, a living condition and sometimes can be unpredictable. Hot or cold weather, too young or too old yeast, too small or too big space in the fermenter for expansion, all affects the fermentation.
Temperature, as said before, have a big effect in the yeast. One of this effects was seen in this project. Yeast strains, even in their living temperature condition, can produce determined substances in certain temperatures. One of this substances is the hydrogen sulfide, a off-flavour. Over high temperature, the yeast stresses and starts to produce a lot of hydrogen sulfide, resulting in a strong smell and taste of rotten egg in the beer.
Another error that destroyed the measurement was the vigorous fermentation. This can cause a clogged airlock with gunk, as seen in Figure 4.16. Sometimes the yeast is so vigorous that the fermentation foam (krausen) is forced into the airlock. If the airlock gets fully clogged and plugged, which was not the case of the Figure 4.16, it can cause a rising of the pressure inside the fermenter causing an explosion of the fermenter in bigger cases or spraying of wort in smaller situations.

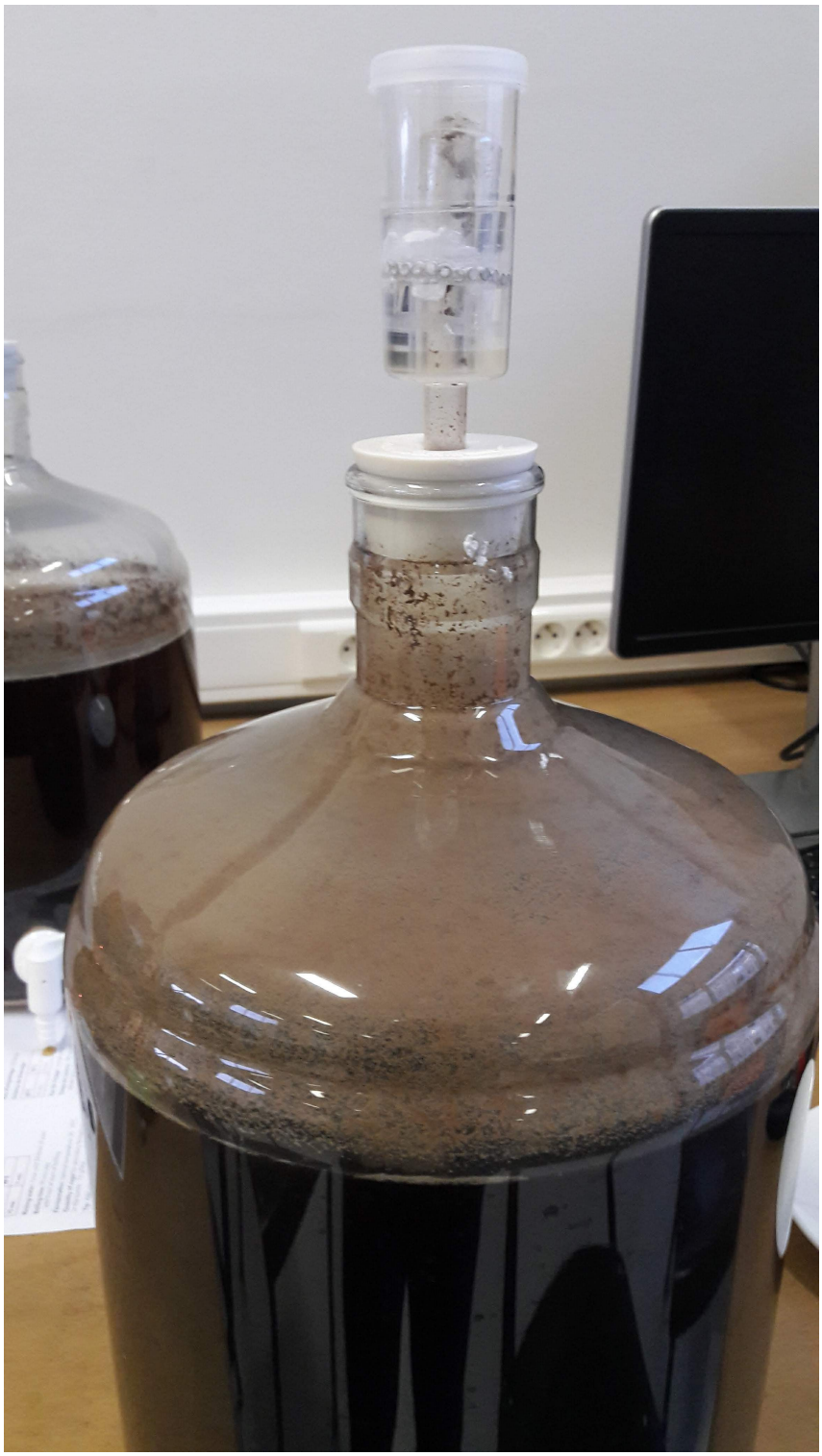


Figure 4.16: Vigorous Fermentation Batch

Chapter 5

Concluding remarks

5.1 Results and discussion

5.1.1 Model

The model developed in this project describes the catabolic reaction of yeast in the fermentation process in batch mode. This new model was created to test if the system could be observable with the measurement available for this project but also to describe the system as simple as it could be.
The assumptions, to make this model as simple as possible, started with the catabolic stoichiometric reaction. It was used the Crabtree effect to ignore the aerobic fermentation, which is a common assumption for fermentation of yeast in beer processes in batch mode. Also, this reaction is in reality a simplification of a system of catabolic reactions, that depends in different types of enzymes, temperatures, pH, compost and concentrations. This simplification is fine for models such as the one applied in this project, that can be seen in several works from the literature, but it is good to know that this complex systems exists so there is a possibility of expansion of the system.
Another assumption is the isobaric system behaviour. The airlock does not give a perfect control of the pressure, as seen in the experimental part, having a switching characteristic more than a steady state one. Also, in critical moments, where there is a malfunctioning of the system, the pressure can drop or increase. So the modelisation of the pressure in the system is something to be consider for future research.
The temperature is also a variable to be considered in a future research for this model. The fermentation process is affected greatly with temperature, and it is possible to not model the temperature variable if there is the assumption that the temperature is well controlled or the system is isothermal, which was not the case for the experimental part of this project.
The last assumption to take a consideration is the lag phase neglection. The bypassing of this phase has a consequence on the experimental analysis and it must be remembered when the measured data is transmitted to the observer. The lag phase is an activation phase of the yeast, and it does not present reactions, so in the model it should be represented as a delay.
A discussion can also be made about the operation mode of the fermentation process. In this model it was considered a batch mode, that means a no inflow nor a outflow of the fermenter. But this operation mode does not perfectly fit in the system because in reality, there is an outflow of the CO_2. Even though, it is the most used operation mode for this process. A fed-batch mode is also used for fermentation processes in the literature, where there is an inflow of substrate in the fermenter and in this case there is a dilution rate associated. This operation mode is interesting for fermentation systems that needs longer fermentation time, meaning more products, but it is rare to use this mode for beer fermentation.
The new model takes into consideration aspects of the representative models presented in Subsection 1.4. Since the parameters were taken from the Article [10], the comparison of these

two models were done. As seen in Figure 5.1, the curves have different velocities probably due to the ethanol concentration that is very small on the new model. Also, the balling based model did not finish the fermentation process in this 10 days of simulation while the new model did, and this is also due to the fact that the ethanol was in small concentration, remembering that ethanol acts as an inhibition actor in the yeast fermentation process due to its poisoning factor.

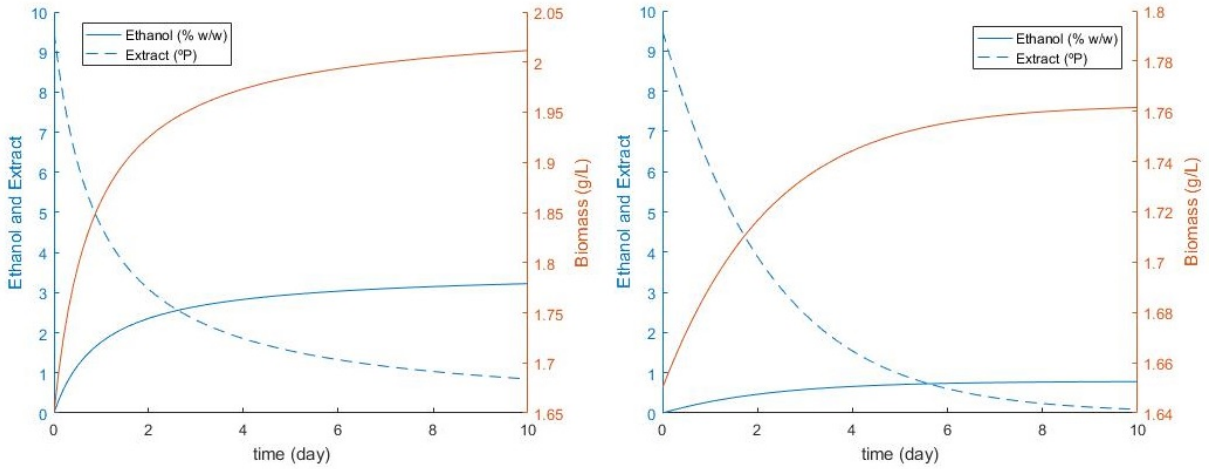


Figure 5.1: Comparison of Models. Left: Balling based model. Right: New model.

5.1.2 Observer

The extended kalman filter is a nice observer for a lot of systems and it is used greatly for similar cases as this project, been optimal for most of them. Even though, it was observed that this non-linear observer looses its observability characteristic along the trajectory.
The analysis of observability was done along the trajectory and it was settled that the system is observable if and only if the substrate concentration is different than zero (The other two variables did not affect the observability matrix), and this was accepted since the absence of substrate means the ending of the reaction, consequently the ending of the fermentation process. The analysis was done at the extremities of the variable values since there was no expectation on observability variability along the trajectory, which might be one of the mistakes.
The lost of observability along the trajectory could be related to the non-linearity aspects of the system combined with the linearisation necessity of the EKF observer. A possible high level of uncertainty due to model linearisation can corroborate with the reason why the system is analytically observable but practically non-observable along the trajectory.
Another possibility is the loss of the observability along the trajectory because of one of the variables. To test this hypothesis, it was done different cases where the measurements would change. So it was tested the case where it was measured CTR with ethanol, CTR with biomass and CTR with substrate and biomass. None of the cases presented better conditions for the loss of observability along the trajectory. Another test was done where a dilution rate was added to CTR to slow down the transfer rate. There was an improvement in observability, meaning that the system lost observability after the usual time. Making it clear that the disappearance of CTR after some days is causing the observability lost.

5.1.3 Experiments

The experimental studies had interesting results for learning the system behaviour but it was not able to develop results for the validation of the model and the observer. To do these validations it is necessary to do more experiments to recover a complete set of data.
Part of the issue for not obtaining the data necessary for validation was the brewing problems, being contamination and temperature the recurrent ones. For the contamination, it was used several chemical resources to solve, it was also changed all the fermenters, none succeed. Two solutions were recommended or change the counter flow chiller or cool the system down, since bacteria prefer high temperature to procreate. Temperature can be resolved by adding a control system for the future project development, which is strongly recommended.
The last CTR sensor version has proven to be most reliable but it was the only one that did not give directly the quantity of emitted bubbles per a fixed time. Because of that, there had to be post acquisition processing that require further research and development. There has to have more analysis on the sensor behaviour, specially on high frequencies, to determine if there is no under sampling in this periods.
A big obstacle of this project experimental part was the union of the acquisition systems, which lead to an abandonment of the use of one of these systems. For future developments of this project, a study needs to be done on this matter or the purchase of more microcontrollers.
For a perspective of this project it is necessary to do more experiments, to not only test the final version of the sensor with both acquisition systems working in parallel but also to acquire enough data to validate the model and the observer.

5.2 Conclusion

The project had the objective of designing a monitoring system for a fermentation process in beer brewing. During its development, one proposed a novel model for the system describing its catabolic reaction. The model was analytically tested and simulated to prove the possibility of the implementation of an observer. The chosen observer was calculated but when simulated it was encountered some errors. In parallel of the theoretical work there was the experimental segment, where two acquisition systems and a sensor were developed. Experiments were done and difficulties were also encountered. To conclude this master thesis, the mishaps on the trajectory were greater leading to inconclusive results but the work done gives a base for future development and a promising perspective.

Glossary

Sign	Description	Unit	Page
$C_6H_{12}O_6$	Glucoses chemical compound		3
C_2H_5OH	Ethanols chemical compound		3
CO_2	Carbon dioxides chemical compound		3–5
O_2	Oxygens chemical compound		3
H_2O	Waters chemical compound		3
X	Biomass concentration	g/L	5, 21, 61, 67
E	Ethanol percentage over weight	% w/w	5, 61, 67
S	Extract or Substrate / Real Extract	°P	5, 6, 21, 61, 67
μ	Specific Growth Rate	day^{-1}	5, 6, 61, 67
q	Specific Product Accumulation	day^{-1}	5, 6, 61
$Y_{X/S}$	Yield of Biomass over substrate		5, 61, 67, 68
$Y_{E/S}$	Yield of Ethanol over substrate		5, 61, 67, 68
μ_{max}	Maximum velocity for biomass production	day^{-1}	6, 61, 67, 68
K_{SX}	Michaelis constant for biomass	°P	6, 61, 67, 68
K_{iX}	Substitution of the Arrhenius constant for biomass	°P	6, 61, 67, 68
q_{Emax}	Maximum velocity for ethanol production	day^{-1}	6, 61, 67, 68
K_{SE}	Michaelis constant for ethanol	% w/w	6, 61, 67

Sign	Description	Unit	Page
K_{iE}	Substitution of the Arrhenius constant for ethanol	% w/w	6, 61, 67
G	Glucose concentration	mol/m^3	7, 8, 16, 64, 65
M	Maltose concentration	mol/m^3	7, 8, 16, 64, 65
N	Maltotriose concentration	mol/m^3	7, 8, 16, 64, 65
X	Yeast concentration	mol/m^3	7, 8, 64, 65
E	Ethanol concentration	mol/m^3	7, 8, 65
T	Temperature	°C	7, 8, 64, 65
μ_1	Specific glucose uptake rate	h^{-1}	7, 8, 16, 64, 65
μ_2	Specific maltose uptake rate	h^{-1}	7, 8, 16, 64, 65
μ_3	Specific maltotriose uptake rate	h^{-1}	7, 8, 16, 64, 65
μ_i	Specific sugar i consumption rate where i = G, M, N	h^{-1}	8, 64
μ_{i0}	Arrhenuis frequency factor	h^{-1}	8, 64
$E_{\mu\ i}$	Arrenhuis activation energy for μ_i	cal/mol	8, 64
R	Gas constant, 1.987	cal/mol K	8, 64
K_i	Michaelis constant for sugar i where i = G, M, N	mol/m^3	8, 64
K_{i0}	Arrhenuis frequency factor for K_i	mol/m^3	8, 64
E_{Ki}	Arrhenius activation energy for K_i	cal/mol	8, 64
K'_i	inhibition constant for sugar i where i = G, M	mol/m^3	8, 64
K'_{i0}	Arrhenuis frequency factor for K_i	mol/m^3	8, 64
E'_{Ki}	Arrhenius activation energy for K'	cal/mol	8, 64
μ_X	Specific yeast growth rate	h^{-1}	8, 65
Y_{XG}	Yield coefficient	mols X per mol G	8, 65
Y_{XM}	Yield coefficient	mols X per mol M	8, 65
Y_{XN}	Yield coefficient	mols X per mol N	8, 65

Sign	Description	Unit	Page
K_X	Empirical yeast growth inhibition constant	$(mol/m^3)^2$	8, 65
X_0	Yeast initial concentration	mol/m^3	8, 65
E_0	Ethanol initial concentration	mol/m^3	8, 65
Y_{EG}	Yield coefficient	mols E per mol G	8, 65
G_0	Glucose initial concentration	mol/m^3	8, 16, 65
Y_{EM}	Yield coefficient	mols E per mol M	8, 65
M_0	Maltose initial concentration	mol/m^3	8, 16, 65
Y_{EN}	Yield coefficient	mols E per mol N	8, 65
N_0	Maltotriose initial concentration	mol/m^3	8, 16, 65
ρ	Density of wort	kg/m^3	8, 65
c_p	Mean heat capacity of wort	J/kg K	8, 65
ΔH_{FG}	Overall heat of fermentation for glucose	J/mol	8, 65
ΔH_{FM}	Overall heat of fermentation for maltose	J/mol	8, 65
ΔH_{FN}	Overall heat of fermentation for maltotriose	J/mol	8, 65
u	Cooling control	$J/m^3/h/^oC$	8, 65
T_c	Effective bulk coolant temperature	^oC	8, 65
X	Biomass Concentration	g/L	10
C_s	Substrate concentration	g/L	10
C_e	Ethanol concentration	g/L	10
C_{dy}	Diacetyl Concentration	ppm	10
C_{ea}	Ethyl Acetate Concentration	ppm	10
X_{lag}	Latent Cells Concentration	g/L	10
X_{act}	Active Cells Concentration	g/L	10
X_{dea}	Dead Cells Concentration	g/L	10
k_S	Substrate yield coefficient		17
S	Substrate concentration	g/L	17, 27
k_E	Ethanol yield coefficient		17
E	Ethanol concentration	g/L	17, 27
k_{CO_2}	Carbon dioxide yield coefficient		17

Sign	Description	Unit	Page
CO_2	Carbon dioxide concentration	g/L	17, 18, 66
k_X	Biomass yield coefficient		17
X	Biomass concentration	g/L	17, 18, 27
μ_X	Biomass specific reaction rate	day^{-1}	17, 18
k_{SX}	Substrate over Biomass yield coefficient		17
k_{EX}	Ethanol over Biomass yield coefficient		17
k_{CO_2X}	Carbon dioxide over Biomass yield coefficient		17, 18
μ_{max}	maximum velocity of biomass production	day^{-1}	17
K_X	Michaelis constant for biomass	g/L	17
k_{iE}	Ethanol inhibition constant	g/L	17
Y_{C_d/CO_2}	Dissolved carbon dioxide over produced carbon dioxide yield coefficient		18
C_d	Dissolved Carbon dioxide concentration	g/L	18
Y_{C_e/CO_2}	Emitted carbon dioxide over produced carbon dioxide yield coefficient		18
C_e	Emitted Carbon dioxide concentration	g/L	18
CTR	Carbon dioxide transfer rate from wort to air	g/L/day	18
$Y_{CO_2/S}$	Yield of Carbon dioxide over substrate		61
SG	Specific Gravity		78
^oP	degrees Plato		78
^oBx	degrees Brix		78

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Appendices

Appendix A

Beer Production and its Nuances

A.1 Ingredients

A.1.1 Water

Water is considered to be the most important ingredient for most Brewers. It composes from 90 to 95% of the beer. Following [35], a brewery must worry about their water providers the same way as their hops or malt. Different water providers have different chemical profiles, so, they give different benefits to different beer styles.
Different chemical profiles give different beer styles so there must be manipulation of the water before the start of the process. The water must be prepared, which means that the brewer will add different chemical compounds, like mineral salts, to adjust the water chemical profile. Following [36], the manipulation of the salts is done more to adjust the pH of the wort because the mineral profile influences more in the conversion of the sugars in it, and there is three salts that are commonly used: sodium, chloride and sulfate. Sodium and chloride change in the sweetness of the beer while the sulfate pushes the bitterness of the hops. Calcium is also important and must be in a minimum of 50ppm.
The minerals can also affect the starch conversion in the wort, but once the sugars are produced, the water composition effect in the flavour of the beer is reduced, but still is presented on the final result, following [1].
[36] also says that alkalinity in the water is avoidable, even though they vary according to the beer styles and the malt acidic composition, mainly low alkalinity is desirable for bright beers and as the color gets darker the alkalinity gets higher and for more acidic mashes.
To resume, brewing water must follow several requirements. It must be potable, transparent, colourless, odourless, free of any strange flavour, follow the chemical profile for the beer style and show absence of nitrates, heavy metals and ammoniac which are harmful for humans.

A.1.2 Malt

Malt are grain seeds that are germinated and then inactivated by heat. The most used malt seed is the Hordeum vulgare, commonly called Barley, but other seeds can also bee used such as wheat, oats, rye, sorghum and millet.[2] says that malt is used as a source of starch and enzymes and in the mashing, this enzymes will break this starches in fermentable sugars which will be used later on by the yeast.
[37] affirms that with a great handling a beer can be created with any grain, but, barley is the heart of the beer in different aspect. In its malted shape, barley contributes with most of the colour, alcohol content and the beer flavour. In less volume, the rest of the grains also contributes with this characteristics, but they cannot be compared with barley.
[36] says that Barley is the ideal grain for beer. This simple grain has a great variety of elements that enables brewers to develop several styles of beers only by playing with its characteristics. Its

seed is a complex package of biochemistry, with specific characteristics, behaviours and necessities which makes a perfect combination for Brewers. It is a full package of starch, proteins, enzymes and other compounds that are perfect for beer making.
There are categories to divide malt, like the way they are dried, how they will be used, their colour, their enzymatic profiles or their amount of toasting. There are some big categories such as base malts, colour malts, caramel & crystal malts, kilned & toasted malts and roasted malts. Base malts are brighter and the main malt on the beer composition because of its high quantity of enzymes for sugar conversion in the mashing. Colour malts are mostly used for adding colour to the beer, since they don't present big enzymatic profiles. Toasted malts are darker and add colour, aroma and flavour but no enzymatic activity to the beer. Caramel & crystal malts are used to give colour and sweetness to the beer, they receive a special treat after malting which crystallize the sugars, as said by [37].

A.1.3 Hops

Hops is the name given to the conic unfertilized flower of the *Humulus lupulus* plant. This species of flowering plant belongs to the *Cannabaceae* family and it is a climbing plant native from the temperate regions of Europe, North America and Asia. To cultivate this flower, the cultivator must do the separation of the male and the female plants because only the female plants that weren't fertilized can generate the conic flower.
J. J. Palmer describes in [1] thate the plants can grow over 20m, their leaves look like grape leaves and their flowers resembles miniature pine cones of 3 to 5cm but light green and as thin as paper. In the base of its petals there are the yellowish lupulin glands that contains the essential oils and essential resins well appreciated by many. The detailed structure of a hop can be seen in the Figure A.1.

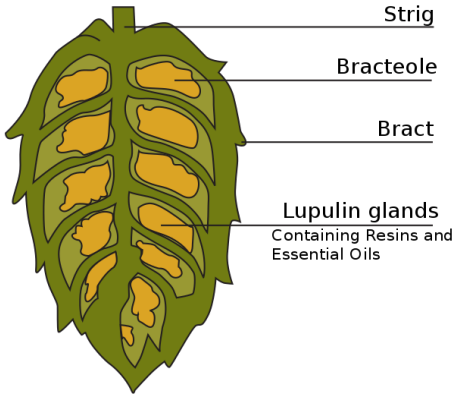

Figure A.1: The anatomy of a Hop [38].

Hops was first used to preserve the beer. It is a natural preservative, and they were directly added at the barrels after fermentation to maintain the freshness while transportation of the beer. Today, it gained other attributes. Hops gives equilibrium to the beer and its presence is so clear that it is considered a signature to some beer styles.
In his book [1], J. J. Palmer explains that the bitterness brought by the hops balance the sweetness of the sugars from the malt and brings a refreshing ending. This bitterness comes from the resin called α acid which is water resistance until it is isomerized by the wort boiling. Bigger the boiling, bigger the isomerization and consequently more bitter the beer will be. But, this process should be done with great caution since the oils that contributes with flavour and aroma characteristics are volatiles and can be lost in this process in great quantity.
There are different varieties of hops that are mainly divided in two groups: bitter and aroma. A. T. Nassif explains in [36] that bitter hops, also called boil hops, are rich in α acids (around

10% of its weight), reason why they are added in the beginning of the boiling and boiled around 1 hour. Aroma hops, also called finishing hops, are added at the end of the boiling, around 15 minutes before its finish, has less α acids (around 5% of its weight), and they give flavour and desired aromas to the beer. There is also a lot of varieties that are in the middle of the both categories which have both characteristics and adding different varieties of hops in different time of the boiling gives the beer a more complex bitter/aroma/flavour profile.

A.1.4 Yeast

Saccharomyces cerevisiae is a species of yeast from the *Fungi* kingdom. It is the micro-organism behind the most common type of fermentation and it is one of the most studied eukaryotic organisms in the biology field. *S. cerevisiae* cells are round to ovoid shaped and their diameters measures $5 - 10\mu m$.
This fungus cells do asexual reproduction by cellular division. They can live and grow regardless of the presence of oxygen, different from most of other organisms. The yeast, can live without oxygen because of the process know as fermentation. Following [1], in the fermentation process, the yeast cells incorporates simple sugars like glucose and maltose and produces carbon dioxide and alcohol as residual products. There are also other residual products, known as by-products like esters, superior alcohols, ketones, phenols and fatty acids.
This products created in the fermentation process can be beneficial or harmful to the beer, affirms J. J. Palmer in [1]. Esters are responsible for the fruity notes, the phenols give spicy notes and medicinal notes in combination with chlorine. Diacetyl, a keton, in small quantity, gives a butter note, desired in strong Pale Ales like Scotch Ales, but it is a very unstable component. When the beer gets older the diacetyl gives a rancid flavour considered a flaw for beers like Light Lagers due to its oxidation. Another undesired by-products are the superior alcohols which are the so-called responsible for the hangovers.
There are two main types of yeast: Ales and Lagers. The old definition would separate them in where the yeast would stay while the process of fermentation, bottom or top, but some strains hinder this separation. So the definition now is based on fermentation temperature. Ales, also known as top-fermenting yeasts prefer high temperatures. Their species name is *Saccharomyces cerevisiae* and they mostly get inactive with temperatures less than 12^{o}C. Lagers, also known as bottom-fermenting yeasts prefer cold temperature. Their species name is *Saccharomyces pastorianus* and some strains can work until 4^{o}C. There is a third yeast group that can create beer called spontaneous or wild, and as its name says, its cells locus are in the wild and so, very rare.
J. J. Palmer describes in [1] that the yeast can be found mainly in two ways: dry and liquid. The dry yeast is dehydrated for conservation propose and must be re-hydrated before use. Both must be maintained in good conditions and temperature controlled. There are also limitations for some specific types of yeast that can only be found or dry or liquid.
The behaviour of the yeast is also an important feature that brewers must be careful. There are three terms brewers use that one must know to understand yeast behaviour: attenuation, flocculation and lag time [36].

- **Attenuation:** Describes the percentage of converted sugars from malt to ethanol and carbon dioxide by yeast action. It is given in percentage and the majority of the yeast attenuates from 65% to 80% in scale. This scale is "apparent" because it is a comparison of the initial and final value of the density of the beer.

- **Flocculation:** Is a phenomena that occurs with yeast where the cells agglutinates in flakes and place themselves in the bottom of the bioreactor after the fermentation process. So, the term, indicates a time and the formation of this flakes, and different yeasts have different flocculation profiles. Some flakes glue in the bottom of the bioreactor, some floats in the

wort and some, that can be highly flocculating, can settle before the fermentation finish and leave high diacetyl and fermentable sugar concentrations in the beer as a consequence.

- **Lag Time:** It is the time lapse between the inoculum and the bubbling from the airlock. A long lag time, more than 24h, can indicates a lack of yeast cells or insufficient aeration.

A.2 Production

A.2.1 Milling

A. T. Nassif says in [36] that milling has a direct impact in the efficiency of the brew where the objective is to expose the endosperm of the grain and separate it from the husk not to grind the malt. A good milling has a proportion of 30% of full husk, 55% of groats, 12% of flour and no full grain [1].
Following [37], milling is when a set of rollers gently smashes the grains, keeping the husks intact to serve as filter material. And it is important that this milling is well done because the endosperm is the starch locus and the starch are the ones that will be destroyed in small sugars to be consumed by the yeast in the fermentation process.

A.2.2 Mashing

Mashing is the act of soak in hot water the barley, which hydrates them, activates the enzymes of the malt and converts the starch of the grains into fermentable sugars. The objective of mashing is to activate the enzymes inside the malt through the cooking of the grains in hot water to convert the starch into fermentable sugars (glucose and maltose) and dextrins that composes the wort.
pH and temperature are fundamental in this process because their combination supports different types of enzymes. Changing their time range, changes the style of the beer produced.
Following [1], the grains have their starch reserves closed in protein/carbohydrate matrices which hinder the physical contact of the enzymes with the starch. Crushing or stirring the grains during mashing helps to hydrate it. Once hydrated, starch can be solubilized, just by heat, or by a combination of heat and enzyme action. In the mash process, 90% of the starches are soluble at 45.5ºC and reach their maximum solubility at 65ºC.
The Figure A.2 shows a typical enzymatic scale in a mashing process. It shows that the enzymes are active in different pH and temperature. A. T. Nassif highlights in **??** that the enzymes can be active outside this boarders, but they will be destroyed as soon as the temperature rises.

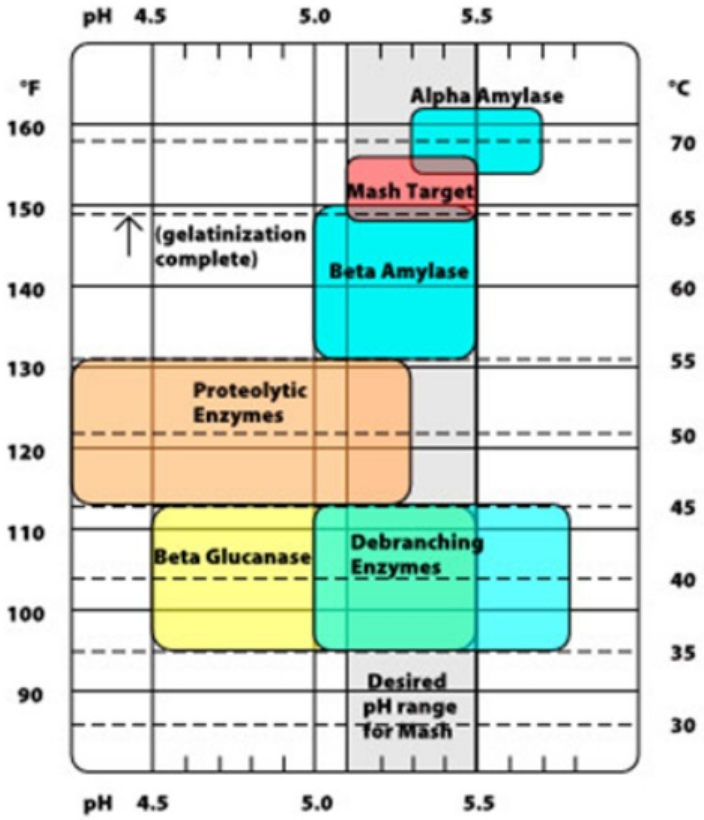

Figure A.2: Typical enzymatic scale in mashing [36].

The mashing process is mainly divided in two big phases: the protein rest and the saccharification. Following [36], both are determined by different enzymatic actions, but mostly by the temperature rates that they occur. The Protein rest occurs from 50 to 55^{o}C. In this phase occurs the big protein breaks into smaller ones and the break of peptides into amino acids. This phase occurs specially when malts have great quantities of protein like wheat or oat. Saccharification occurs from 55 to 72^{o}C. In this phase occurs the break of the bigger sugars into smaller sugars. This breakage is done by two enzymes: *alpha-amylase* and *beta-amylase*.

- ***beta-amylase*:** This enzymes are active in temperatures from 55 to 65^{o}C and pH between 5.0 and 5.5. They breaks the linear starch chains into fermentable sugars. If this enzymes work more (more time in this temperature step), there is more fermentable sugars in the wort, meaning an increase of the attenuation, more alcohol and smaller beer body [37].
- ***alpha-amylase*:** This enzymes are active in temperature from 68 to 72^{o}C and pH between 5.3 and 5.7. R. Mosher states in [37] that enzymes break chains of amylose or amylopectin randomly, generating smaller sugars (more fermentable) but also bigger sugars (less fermentable or non-fermentable). If the mashing is done more in the temperature of the alpha-amylases, the wort will have more diversity of sugars, meaning a sweeter beer, with a stronger body and less alcoholic.

A.2.3 Boiling

The boiling will mostly happen in an hour. In this process several important things happen, R. Mosher in his book *Mastering Homebrew* [37] lists all the things that happens in the boiling. This list can be seen in the Figure **??** and it summarizes this process.

- Pasteurization of the wort
- Cessation of any remaining enzyme activity
- Isomerization and dissolving of hop bittering compounds
- The removal of unwanted aromatic compounds, especially DMS, but also some unpleasant hop and roasted malt volatiles
- Coagulation of excess long-chain proteins, aided by hop tannins
- Incorporation of hop aromas through different mechanisms

Figure A.3: What happens in the kettle [37].

A.3 Representative Dynamic Models Summary

A.3.1 Balling Based Model

A.3.1.1 ODE Model

Yields of the chemical compounds:

$$Y_{\mathrm{E/S}} = \frac{1.000g}{2.0665g} = 0.4839 \tag{A.1}$$

$$Y_{\mathrm{X/S}} = \frac{0.11g}{2.0665g} = 0.0532 \tag{A.2}$$

$$Y_{\mathrm{CO_2/S}} = \frac{0.9565g}{2.0665g} = 0.4628 \tag{A.3}$$

Biomass:

$$\frac{dX}{dt} = \mu(t)X(t) \tag{A.4}$$

Ethanol:

$$\frac{dE}{dt} = q(t)X(t) \tag{A.5}$$

Extract:

$$\frac{dS}{dt} = -\frac{1}{Y_{\mathrm{X/S}}}\frac{dX}{dt} - \frac{1}{Y_{\mathrm{E/S}}}\frac{dE}{dt} \tag{A.6}$$

§1 Specific growth rate and product accumulation rate:

Monod:

$$\mu(t) = \mu_{\max}\frac{S(t)}{K_{\mathrm{SX}} + S(t)} \tag{A.7}$$

$$q(t) = q_{\mathrm{Emax}}\frac{S(t)}{K_{\mathrm{SE}} + S(t)} \tag{A.8}$$

Aiba:

$$\mu(t) = \mu_{\max}\frac{S(t)}{K_{\mathrm{SX}} + S(t)}e^{-K_{\mathrm{iX}}E} \tag{A.9}$$

$$q(t) = q_{\mathrm{Emax}}\frac{S(t)}{K_{\mathrm{SE}} + S(t)}e^{-K_{\mathrm{iE}}E} \tag{A.10}$$

Tessier:

$$\mu(t) = \mu_{\max}(1 - e^{-\frac{S(t)}{K_{\mathrm{SX}}}}) \tag{A.11}$$

$$q(t) = q_{\mathrm{Emax}}(1 - e^{-\frac{S(t)}{K_{\mathrm{SE}}}}) \tag{A.12}$$

A.3.1.2 Algebraic Linear Model

Substrate:

$$S(t) = S_{int} - aCO_2(t) \tag{A.13}$$

Ethanol:

$$E(t) = b(S_{int} - S(t)) \tag{A.14}$$

Biomass:

$$X(t) = 0.1[S(t)_{i-1} - S(t)_i] \tag{A.15}$$

A.3.1.3 Simulation of different kinetic models

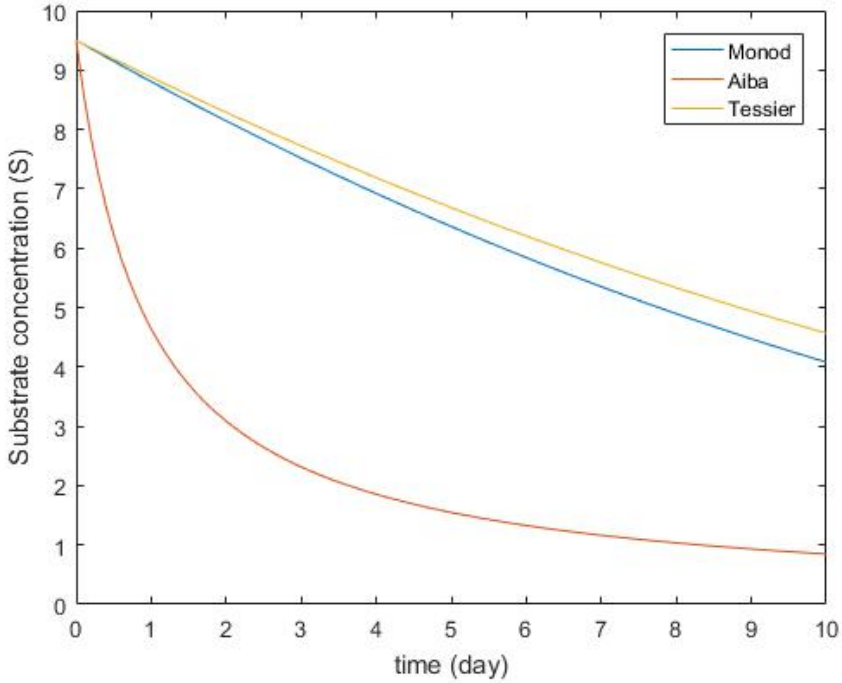

Figure A.4: Substrate Consumption

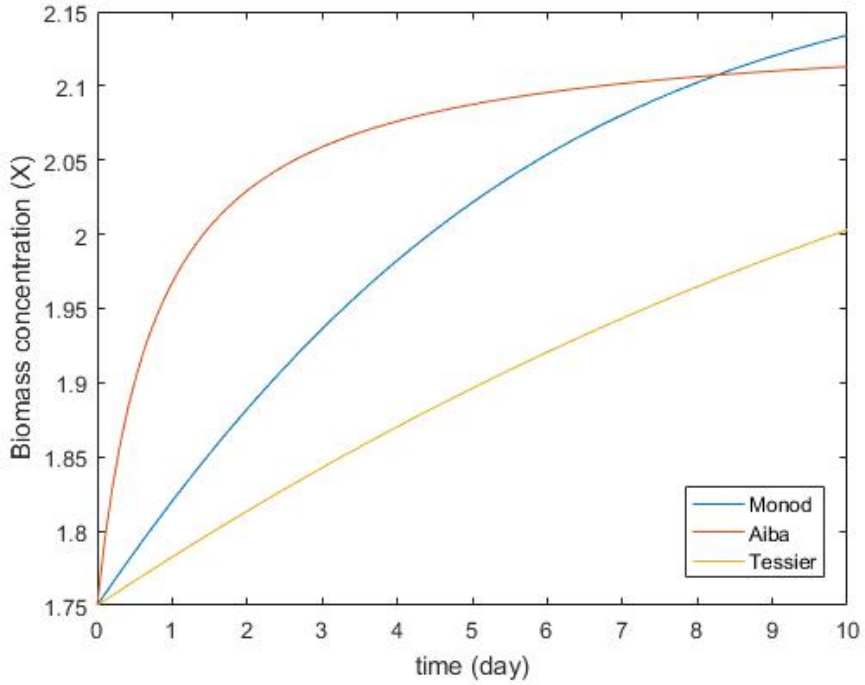

Figure A.5: Biomass production

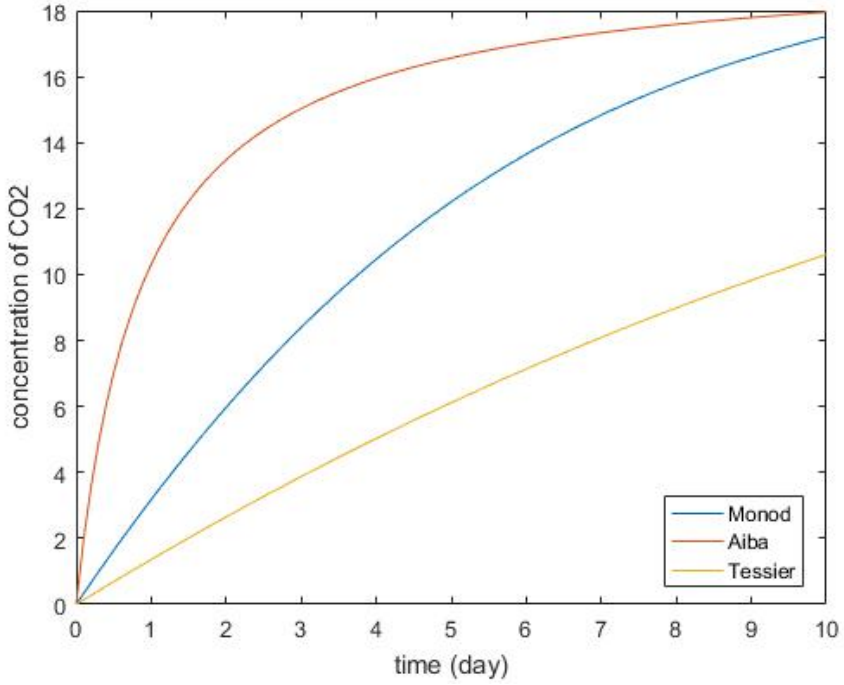

Figure A.6: CO2 Production

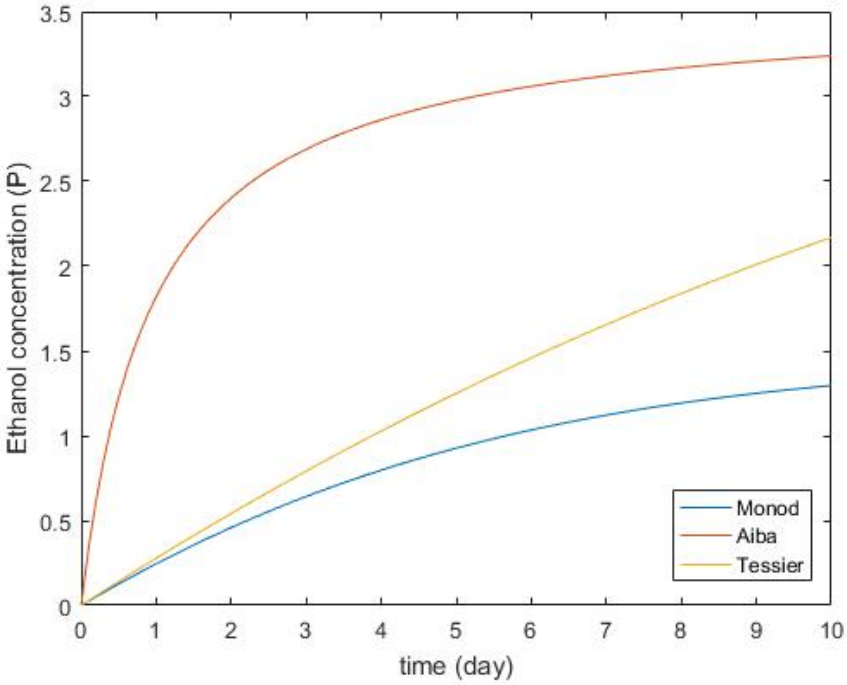

Figure A.7: Ethanol Production

A.3.2 Ramirez & Gee Model

Glucose:

$$\frac{dG}{dt} = -\mu_1 \; X \tag{A.16}$$

Maltose:

$$\frac{dM}{dt} = -\mu_2 \; X \tag{A.17}$$

Maltotriose:

$$\frac{dN}{dt} = -\mu_3 \; X \tag{A.18}$$

Specific growth rate:

$$\mu_1 = \frac{\mu_G \; G}{K_G + G} \tag{A.19}$$

$$\mu_2 = \frac{\mu_M \; M}{K_M + M} \; \frac{K'_G}{K'_G + G} \tag{A.20}$$

$$\mu_3 = \frac{\mu_N \; N}{K_N + N} \; \frac{K'_G}{K'_G + G} \; \frac{K'_M}{K'_M + M} \tag{A.21}$$

Maximum reaction velocities:

$$\mu_{\mathrm{i}} = \mu_{\mathrm{i0}} \; e^{-\frac{\mathrm{E}_{\mu\,\mathrm{i}}}{RT}} \;\; , \;\; i = G, M, N \tag{A.22}$$

Michaelis constants:

$$K_{\mathrm{i}} = K_{\mathrm{i0}} \; e^{-\frac{E_{\mathrm{Ki}}}{RT}} \;\; , \;\; i = G, M, N \tag{A.23}$$

Inhibition constants:

$$K'_{\mathrm{i}} = K'_{\mathrm{i0}} \; e^{-\frac{E'_{\mathrm{Ki}}}{RT}} \;\; , \;\; i = G, M \tag{A.24}$$

Biomass:

$$\frac{dX}{dt} = \mu_{\mathrm{X}}\ X \tag{A.25}$$

Specific Groth rate for Biomass:

$$\mu_{\mathrm{X}} = (Y_{\mathrm{XG}}\ \mu_1 + Y_{\mathrm{XM}}\ \mu_2 + Y_{\mathrm{XN}}\ \mu_3)\ \frac{K_{\mathrm{X}}}{K_{\mathrm{X}} + (X - X_0)^2} \tag{A.26}$$

Ethanol:

$$E = E_0 + Y_{\mathrm{EG}}(G_0 - G) + Y_{\mathrm{EM}}(M_0 - M) + Y_{\mathrm{EN}}(N_0 - N) \tag{A.27}$$

Temperature:

$$\frac{dT}{dt} = \frac{1}{\rho c_{\mathrm{p}}}\left(\Delta\ \mathrm{H_{FG}}\frac{dG}{dt} + \Delta\ \mathrm{H_{FM}}\frac{dM}{dt} + \Delta\ \mathrm{H_{FN}}\frac{dN}{dt} - u(T - T_{\mathrm{c}})\right) \tag{A.28}$$

A.3.3 Andrés-Toro model

$$X_{act}(0) + X_{lag}(0) = 0.5\ X_{inc}(0),\ for\ t\ <\ t_{lag} \tag{A.29}$$

Temperature:

$$\mu_{i_0} = A\ e^{B/RT} \tag{A.30}$$

A.3.3.1 Lag phase

$$X_{sus} = X_{act}(t) + X_{lag}(t) + X_{dea}(t) \tag{A.31}$$

$$\frac{dX_{sus}(t)}{dt} = -\mu_{SD}(X_{sus}(t) - 0.5\ X_{inc}),\ for\ t\ <\ t_{lag} \tag{A.32}$$

Where:

$$\mu_{SD} = \frac{0.5\ \mu_{SD_0}\ C_{S_0}}{0.5\ C_{S_0} + C_e(t)} \tag{A.33}$$

$$\frac{dX_{act}(t)}{dt} = \mu_{lag}\ X_{lag}(t) = \mu_L(0.5\ X_{inc} - X_{act}(t)),\ for\ t\ <\ t_{lag} \tag{A.34}$$

A.3.3.2 Fermentation phase

Active Cells:

$$\frac{dX_{act}(t)}{dt} = \mu_x\ X_{act}(t) + \mu_L\ X_{lag}(t) - \mu_{DT}\ X_{act}(t),\ for\ t\ >\ t_{lag} \tag{A.35}$$

$$\mu_x = \frac{\mu_{x_0}\ C_s(t)}{k_x + C_e(t)} \tag{A.36}$$

Substrate:

$$\frac{dC_s(t)}{dt} = -\mu_s\ X_{act}(t) \tag{A.37}$$

$$\mu_s = \frac{\mu_{s_0}\ C_s(t)}{k_s + C_s(t)} \tag{A.38}$$

Ethanol:

$$\frac{dC_e(t)}{dt} = f\ \mu_e\ X_{act}(t) \tag{A.39}$$

$$f = 1 - \frac{C_e(t)}{0.5\ C_{s_0}} \tag{A.40}$$

$$\mu_e = \frac{\mu_{e_0}\ C_s(t)}{k_e + C_s(t)} \tag{A.41}$$

Appendix B

New Model extras

B.1 Validation of CO_2 system of equations

For checking if the equations of produced CO_2 (Equations 2.15 and 2.16) are valid, it is possible to differentiate the Equation 2.14 over time and see if the function is true.

$$\frac{dCO_2}{dt} = \frac{dC_d}{dt} + \frac{dC_e}{dt} \tag{B.1}$$

$$\frac{dCO_2}{dt} = Y_{C_d/CO_2}\ \frac{dCO_2}{dt} - CTR + Y_{C_e/CO_2}\ \frac{dCO_2}{dt} + CTR \tag{B.2}$$

$$\frac{dCO_2}{dt} = (Y_{C_d/CO_2} + Y_{C_e/CO_2})\frac{dCO_2}{dt}\ \rightarrow\ \frac{dCO_2}{dt} = 1 \times \frac{dCO_2}{dt} \tag{B.3}$$

B.2 ODE System Summary

ODE system
$\dot{S} = -k_{SX}\ \mu_X X$ $\dot{X} = \mu_X X$ $\dot{E} = k_{EX}\ \mu_X X$
Reaction Rates
$\mu_X = \mu_{max}\frac{S}{K_X+S}\ e^{-k_{iE}\ E}$
CO_2 **measurement**
$CTR = Y_{C_d/CO_2}\ k_{CO_2X}\ \mu_X X$

Table B.1: Table of ODE System

B.3 Parameter conversion

The Parameters for this model were based on the article [10]. For visualization propose, the new model parameters and functions will always be in the left part of the equations while the right part will be reserved for the balling model one. To begin, it is compared the biomass parameters.

$$\dot{X} = \mu_X X \iff \frac{dX}{dt} = \mu(t)X(t) \tag{B.4}$$

So, using the Aiba kinetics model (B.5) μ_{max} will be equal to $\mu_{\max}$, K_X to $\mathrm{K_{SX}}$ and k_{iE} to $\mathrm{K_{iX}}$.

$$\mu_X = \mu_{max}\frac{S}{K_X + S}\ e^{-k_{iE}\ E} \iff \mu(t) = \mu_{\max}\frac{S(t)}{K_{\mathrm{SX}} + S(t)}e^{-K_{\mathrm{iX}}E} \tag{B.5}$$

For the Ethanol, one has:

$$\dot{E} = k_{EX}\ \mu_X X \iff \frac{dE}{dt} = q(t)X(t) \tag{B.6}$$

$$k_{EX}\ \mu_{max}\frac{S}{K_X + S}\ e^{-k_{iE}\ E} \iff q_{\mathrm{Emax}}\frac{S(t)}{K_{\mathrm{SE}} + S(t)}e^{-K_{\mathrm{iE}}E} \tag{B.7}$$

Considering $K_{SX} = K_{SE}$ and $K_{iX} = K_{iE}$ than $k_{EX} = q_{E_{max}}/\mu_{max}$.
The substrate signal parameters will be calculated as follow.

$$\dot{S} = -k_{SX}\ \mu_X X \iff \frac{dS}{dt} = -\frac{1}{Y_{\mathrm{X/S}}}\frac{dX}{dt} - \frac{1}{Y_{\mathrm{E/S}}}\frac{dE}{dt} \tag{B.8}$$

$$\dot{S} = -k_{SX}\ \mu_X X \iff \frac{dS}{dt} = -\left(\frac{1}{Y_{\mathrm{X/S}}}\mu + \frac{1}{Y_{\mathrm{E/S}}}q\right)X \tag{B.9}$$

To obtain the value of k_{SX} it is assumed the same assumption of the ethanol where $K_{SX} = K_{SE}$ and $K_{iX} = K_{iE}$ so:

$$k_{SX}\ \mu_{max}\frac{S}{K_X + S}\ e^{-k_{iE}\ E} \iff (\frac{1}{Y_{\mathrm{X/S}}}\mu_{\max} + \frac{1}{Y_{\mathrm{E/S}}}q_{\mathrm{Emax}})\frac{S(t)}{K_{\mathrm{SX}} + S(t)}e^{-K_{\mathrm{iX}}E} \tag{B.10}$$

$$k_{SX} \iff \left(\frac{1}{Y_{\mathrm{X/S}}} + \frac{1}{Y_{\mathrm{E/S}}}\frac{q_{\mathrm{Emax}}}{\mu_{max}}\right) \tag{B.11}$$

The last two parameters that are missing are the Y_{C_d/CO_2} and k_{CO_2X} which comes from the measurement of CTR. Since there is not enough experiments to conduct a parametrization for this two, and the [10] does not apply CO_2 into its ODE, they were treated as one parameter and were calculated as a fraction of the ethanol production. It was also used the yields of the Balling equation to help with the value.

$$Y_{C_d/CO_2}\ k_{CO_2X} \iff \frac{1}{Y_{CO_2/S}}Y_{E/CO_2}\frac{q_{\mathrm{Emax}}}{\mu_{max}} \tag{B.12}$$

B.3.1 Summary

New Model	**Balling Model** [10]
μ_{max}	$\mu_{\max}$
K_X	$\mathrm{K_{SX}}$
k_{iE}	$\mathrm{K_{iX}}$
k_{EX}	$\frac{q_{Emax}}{\mu_{max}}$
k_{SX}	$\left(\frac{1}{Y_{\mathrm{X/S}}} + \frac{1}{Y_{\mathrm{E/S}}}\frac{q_{\mathrm{Emax}}}{\mu_{max}}\right)$
$Y_{C_d/CO_2}\ k_{CO_2X}$	$\frac{1}{Y_{CO_2/S}} Y_{E/CO_2} \frac{q_{\mathrm{Emax}}}{\mu_{max}}$

Table B.2: Parameters

Appendix C

Mathematical developments

C.1 Jacobian matrix

Presented in 1841 by Carl Gustav Jacob Jacobi [39], firstly called functional determinants, the Jacobian matrix C.1 is composed by all first-order partial derivatives ($\mathrm{J_{ij}} = \frac{\partial \mathrm{f_i}}{\partial \mathrm{x_j}}$) of a vector-valued function ($\mathrm{f_1}, \mathrm{f_2}, ..., \mathrm{f_m}$) with respect to all variables ($\mathrm{x_1}, \mathrm{x_2}, ..., \mathrm{x_n}$).

$$J = \begin{bmatrix} \frac{\partial f_1}{\partial x_1} & \cdots & \frac{\partial f_1}{\partial x_n} \\ \vdots & \ddots & \vdots \\ \frac{\partial f_m}{\partial x_1} & \cdots & \frac{\partial f_m}{\partial x_n} \end{bmatrix} \tag{C.1}$$

For the use of the LTI system observability method the Jacobian have a great role. This matrix is the one used to linearize nonlinear state equations. In the state space representation, the state matrix A will be a Jacobian matrix of the functions $\mathrm{f_1}, \mathrm{f_2}, ..., \mathrm{f_m}$ by the state variables $\mathrm{x_1}, \mathrm{x_2}, ..., \mathrm{x_n}$.

C.2 Detailed Elements of the Observability Matrices

C.2.1 Measurement of Biomass

$$C = [1\ 0\ 0] \tag{C.2}$$

$$CA = \begin{bmatrix} \frac{\mu_{max} S}{S + K_{sx}} & 0 & \frac{\mu_{max} K_{sx} X}{(S + K_{sx})^2} \end{bmatrix} \tag{C.3}$$

$$CA^2 = \begin{bmatrix} CA_1^2 & 0 & CA_3^2 \end{bmatrix} \tag{C.4}$$

Where:

$$CA_1^2 = \frac{(S\mu_{max})^2}{(K_{sx} + S)^2} + \frac{X\mu_{max}K_{sx}}{(K_{sx} + S)^2} \left(\frac{-S\mu_{max}}{Y_{xs}(K_{sx} + S)} - \frac{Sq_{max}}{Y_{ps}(K_{sp} + S)} \right) \tag{C.5}$$

$$CA_3^2 = \frac{X\mu_{max}K_{sx}}{(K_{sx} + S)^2} \left[\frac{S\mu_{max}}{K_{sx} + S} - \frac{X\mu_{max}K_{sx}}{Y_{xs}(K_{sx} + S)^2} - \frac{Xq_{max}K_{sp}}{Y_{ps}(K_{sp} + S)^2} \right] \tag{C.6}$$

C.2.2 Measurement of Ethanol

$$O_{21} = \frac{Sq_{max}}{K_{sp} + S} \tag{C.7}$$

$$O_{23} = \frac{Xq_{max}K_{sp}}{(K_{sp} + S)^2} \tag{C.8}$$

$$O_{31} = \frac{S\mu_{max}Sq_{max}}{(K_{sx}+S)(K_{sp}+S)} + \frac{Xq_{max}K_{sp}}{(K_{sp}+S)^2}\left[\frac{-S\mu_{max}}{Y_{xs}(K_{sx}+S)} - \frac{Sq_{max}}{Y_{ps}(K_{sp}+S)}\right] \tag{C.9}$$

$$O_{33} = \frac{X\mu_{max}K_{sx}Sq_{max}}{(K_{sx}+S)^2(K_{sp}+S)} + \frac{Xq_{max}K_{sp}}{(K_{sp}+S)^2}\left[-\frac{X\mu_{max}K_{sx}}{Y_{xs}(K_{sx}+S)^2} - \frac{Xq_{max}K_{sp}}{Y_{ps}(K_{sp}+S)^2}\right] \tag{C.10}$$

C.2.3 Measurement of Substrate

$$C = [0\ 0\ 1] \tag{C.11}$$

$$CA = \begin{bmatrix} \frac{-S\mu_{max}}{Y_{xs}(K_{sx}+S)} - \frac{Sq_{max}}{Y_{ps}(K_{sp}+S)} & 0 & -\frac{X\mu_{max}K_{sx}}{Y_{xs}(K_{sx}+S)^2} - \frac{Xq_{max}K_{sp}}{Y_{ps}(K_{sp}+S)^2} \end{bmatrix} \tag{C.12}$$

$$CA^2 = \begin{bmatrix} CA_1^2 & 0 & CA_2^2 \end{bmatrix} \tag{C.13}$$

Where:

$$\begin{aligned} CA_1^2 &= (\frac{S\mu_{max}}{K_{sx}+S})(\frac{-S\mu_{max}}{Y_{xs}(K_{sx}+S)} - \frac{Sq_{max}}{Y_{ps}(K_{sp}+S)}) \\ &+ (-\frac{X\mu_{max}K_{sx}}{Y_{xs}(K_{sx}+S)^2} - \frac{Xq_{max}K_{sp}}{Y_{ps}(K_{sp}+S)^2})(\frac{-S\mu_{max}}{Y_{xs}(K_{sx}+S)} - \frac{Sq_{max}}{Y_{ps}(K_{sp}+S)}) \end{aligned} \tag{C.14}$$

$$CA_3^2 = (\frac{-S\mu_{max}}{Y_{xs}(K_{sx}+S)} - \frac{Sq_{max}}{Y_{ps}(K_{sp}+S)})(\frac{X\mu_{max}K_{sx}}{(K_{sx}+S)^2}) + (-\frac{X\mu_{max}K_{sx}}{Y_{xs}(K_{sx}+S)^2} - \frac{Xq_{max}K_{sp}}{Y_{ps}(K_{sp}+S)^2})^2 \tag{C.15}$$

C.3 Echelon form transformation of the Observability Matrix when Ethanol is measured for Balling based Model

$$O = \begin{bmatrix} 0 & 1 & 0 \\ O_{21} & 0 & O_{23} \\ O_{31} & 0 & O_{33} \end{bmatrix} \xrightarrow{C_1 \rightleftharpoons C_2} \begin{bmatrix} 1 & 0 & 0 \\ 0 & O_{21} & O_{23} \\ 0 & O_{31} & O_{33} \end{bmatrix} \tag{C.16}$$

$$O = \begin{bmatrix} 1 & 0 & 0 \\ 0 & O_{21} & O_{23} \\ 0 & O_{31} & O_{33} \end{bmatrix} \xrightarrow{L_2 = \frac{L_2}{O_{21}}} \begin{bmatrix} 1 & 0 & 0 \\ 0 & 1 & \frac{O_{23}}{O_{21}} \\ 0 & O_{31} & O_{33} \end{bmatrix} \tag{C.17}$$

$$O = \begin{bmatrix} 1 & 0 & 0 \\ 0 & 1 & \frac{O_{23}}{O_{21}} \\ 0 & O_{31} & O_{33} \end{bmatrix} \xrightarrow{L_3 = L_3 - (L_2)(O_{31})} \begin{bmatrix} 1 & 0 & 0 \\ 0 & 1 & \frac{O_{23}}{O_{21}} \\ 0 & 0 & O_{33} - \frac{O_{23}O_{31}}{O_{21}} \end{bmatrix} \tag{C.18}$$

$$O = \begin{bmatrix} 1 & 0 & 0 \\ 0 & 1 & \frac{O_{23}}{O_{21}} \\ 0 & 0 & O_{33} - \frac{O_{23}O_{31}}{O_{21}} \end{bmatrix} \xrightarrow{L_3 = \frac{L_3}{L_3}} \begin{bmatrix} 1 & 0 & 0 \\ 0 & 1 & \frac{O_{23}}{O_{21}} \\ 0 & 0 & 1 \end{bmatrix} \tag{C.19}$$

C.4 Observability mathematical analyses for Ethanol measurement

First, lets analyse the denominator of 2.31. Since $O_{21} = 0$ would be a mathematical blurring, so O_{21} can never be equal to 0 and that generates the C.20. It is good to remind that the parameters μ_{max}, q_{max}, K_{sx}, K_{sp}, Y_{xs} and Y_{ps} are always positive, time invariant and different then 0 so the analysis will be in the function of S.

$$O_{21} \neq 0 \rightarrow \frac{S\ q_{max}}{K_{sp} + S} \neq 0 \tag{C.20}$$

Doing the limit analysis (C.21) of C.20 it is clear that, for the system to be observable the substrate concentration cannot be equals to zero. This condition is true because the substrate will never be fully consumed and even though it tends to zero it will never be zero. also, the limit test where the parameter S goes to infinite is not applied due to the physical aspect of the project.

$$\lim_{S \to 0} \frac{S\ q_{max}}{K_{sp} + S} = 0 \tag{C.21}$$

The second condition comes from the numerator of 2.31 where $O_{33}O_{21} - O_{23}O_{31}$ must be different than 0 because a number, different than 0, divided by zero will give zero. Again, we must consider that the parameters μ_{max}, q_{max}, K_{sx}, K_{sp}, Y_{xs} and Y_{ps} are always positive, time invariant and different then 0.

$$O_{33}O_{21} - O_{23}O_{31} \neq 0 \tag{C.22}$$

$$\begin{aligned} &\frac{Xq_{max}K_{sp}}{(K_{sp}+S)^2}\frac{Sq_{max}}{K_{sp}+S} - \\ &\left\{\frac{X\mu_{max}K_{sx}Sq_{max}}{(K_{sx}+S)^2(K_{sp}+S)} + \frac{Xq_{max}K_{sp}}{(K_{sp}+S)^2}\left[-\frac{X\mu_{max}K_{sx}}{Y_{xs}(K_{sx}+S)^2} - \frac{Xq_{max}K_{sp}}{Y_{ps}(K_{sp}+S)^2}\right]\right\} \\ &\left\{\frac{S\mu_{max}Sq_{max}}{(K_{sx}+S)(K_{sp}+S)} + \frac{Xq_{max}K_{sp}}{(K_{sp}+S)^2}\left[\frac{-S\mu_{max}}{Y_{xs}(K_{sx}+S)} - \frac{Sq_{max}}{Y_{ps}(K_{sp}+S)}\right]\right\} \neq 0 \end{aligned} \tag{C.23}$$

After some mathematical manipulation of C.23, the function becomes a multiplication like ab $\neq 0$ so, neither a (C.24) or b (C.25) can be equal to 0.

$$a = \frac{XSq_{max}}{(K_{sx}+S)^2(K_{sp}+S)^5Y_{xs}Y_{ps}} \neq 0 \tag{C.24}$$

$$\begin{aligned} b = {} & \mu_{max}K_{sx}Y_{xs}Y_{ps}(K_{sp}+S)^4 \\ & - Xq_{max}K_{sp}(\mu_{max}K_{sx}Y_{ps}(K_{sp}+S)^2 + q_{max}K_{sp}Y_{xs}(K_{sx}+S)^2) \ - \ K_{sp}q_{max}(K_{sx}+S) \\ & \left[S\mu_{max}Y_{xs}Y_{ps}(K_{sp}+S)^2 - X\mu_{max}K_{sp}Y_{ps}(K_{sp}+S) - Xq_{max}K_{sp}Y_{xs}(K_{sx}+S)\right] \neq 0 \end{aligned} \tag{C.25}$$

For b (C.25) it needs more mathematical manipulations, but for a (C.24) it is possible to analyze. Following the same type of analysis of C.20 neither S or X will tend to the infinite, it is a physical impossibility, also, following the chemical reaction of the system their is a direct relationship between S and X and when the substrate is decomposed the biomass is created. So, if the substrate goes to zero, the biomass goes to its maximum value and vice versa.

$$\lim_{S \to 0} \frac{XSq_{max}}{(K_{sx}+S)^2(K_{sp}+S)^5Y_{xs}Y_{ps}} = 0 \tag{C.26}$$

$$\lim_{X \to 0} \frac{XSq_{max}}{(K_{sx}+S)^2(K_{sp}+S)^5Y_{xs}Y_{ps}} = 0 \tag{C.27}$$

The limit test provided that S and X cannot be zero. As said before, the substrate never goes to zero, but the biomass starts in zero. Even though that, the biomass is only at the first iteration equals to zero because, again, it is formed during the chemical reaction, so this does not prove that the system is not observable.
Continuing the mathematical manipulation of b (C.25) it will end on a big sum equation, so it was created some auxiliary constants to easy the observation. So C.28 is what will be analysed and C.29 is the list of the auxiliary constants.

$$\begin{aligned} &aux_1 + S(4aux_2 - aux_3) + S^2(6aux_4 - aux_5 - 2aux_6) + S^3(4aux_7 - 2aux_8 - aux_9) \\ &+ S^4(aux_{10} - aux_{11}) + XS(-aux_{12} + aux_{13}) + XS^2(aux_{14} - aux_{15}) \neq 0 \end{aligned} \tag{C.28}$$

$$\begin{aligned} aux_1 &= \mu_{max} K_{sx} K_{sp}^4 Y_{sx} Y_{ps} \\ aux_2 &= \mu_{max} K_{sx} K_{sp}^3 Y_{sx} Y_{ps} \\ aux_3 &= \mu_{max} q_{max} K_{sx} K_{sp}^3 Y_{sx} Y_{ps} \\ aux_4 &= \mu_{max} K_{sx} K_{sp}^2 Y_{sx} Y_{ps} \\ aux_5 &= \mu_{max} q_{max} K_{sp}^3 Y_{sx} Y_{ps} \\ aux_6 &= \mu_{max} q_{max} K_{sx} K_{sp}^2 Y_{sx} Y_{ps} \\ aux_7 &= \mu_{max} K_{sx} K_{sp} Y_{sx} Y_{ps} \\ aux_8 &= \mu_{max} q_{max} K_{sp}^2 Y_{sx} Y_{ps} \\ aux_9 &= \mu_{max} q_{max} K_{sx} K_{sp} Y_{sx} Y_{ps} \\ aux_{10} &= \mu_{max} K_{sx} Y_{sx} Y_{ps} \\ aux_{11} &= \mu_{max} q_{max} K_{sp} Y_{sx} Y_{ps} \\ aux_{12} &= \mu_{max} q_{max} K_{sx} K_{sp}^2 Y_{ps} \\ aux_{13} &= \mu_{max} q_{max} K_{sp}^3 Y_{ps} \\ aux_{14} &= \mu_{max} q_{max} K_{sp}^2 Y_{ps} \\ aux_{15} &= \mu_{max} q_{max} K_{sx} K_{sp} Y_{ps} \end{aligned} \tag{C.29}$$

Observing C.28 there is an independent constant in the equation (aux_1), so even if S and X are equal to zero, the equation will never be zero.

C.5 Simple Model Observability test: Numerical

The first step is to extract several points that represents the values of each state variable along the trajectory like in C.1.

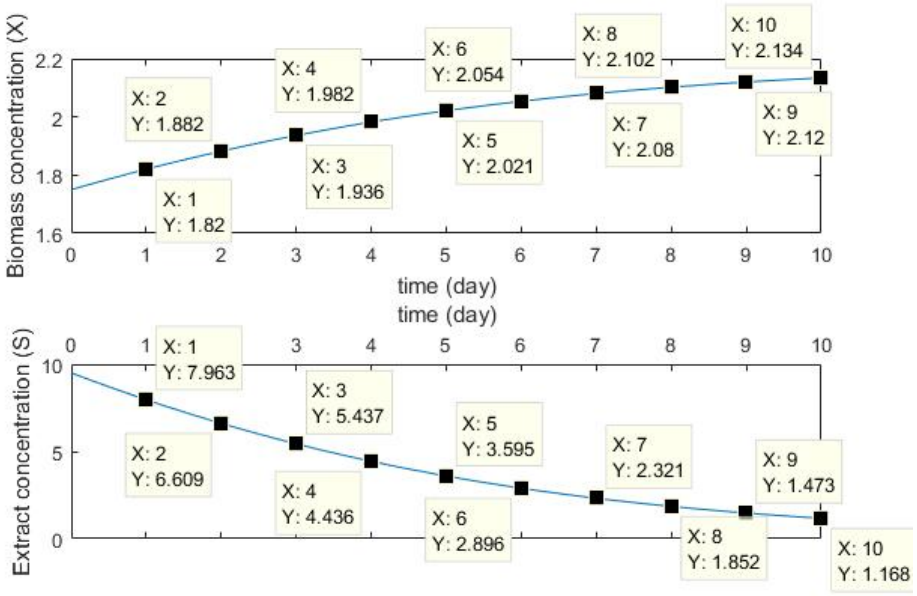

Figure C.1: Extract and Biomass concentrations over time with chosen points along the trajectory

Then, it is calculated the Jacobian matrix 2.27 and after it, for every chosen point, it is replaced the values of the points and parameters so it is possible to find eigenvalues C.30 and eigenvectors C.31.

$$\lambda_{1,2,3} = \begin{bmatrix} 0 \\ -0.562 \\ 5.57 * 10^{-5} \end{bmatrix} \tag{C.30}$$

$$V = \begin{bmatrix} 0 & -0.0463 & -2.73 \\ 1 & -0.154 & 24.6 \\ 0 & 1 & 1 \end{bmatrix} \tag{C.31}$$

Proceeding with the calculation, it is mandatory to chose an output matrix C that will represent which signal it is considered as an output state variable, which will be measured. The ideal is to check all possibilities, considering that all signals could be measured, so first it was chosen to check every signal separately, and after, their combinations.
The Diagonal form comes right after. This form is calculated so it would be possible to analyse the observability matrix and it is a great visual way to analyse if the observability test is working properly. Also, it is only necessary to calculate the state matrix C.32 and the output matrix C.33.

$$A_p = \begin{bmatrix} 0 & 0 & 0 \\ 1 & -0.562 & 0 \\ 0 & 0 & 0 \end{bmatrix} \tag{C.32}$$

$$C_p = \begin{bmatrix} 0 & 1 & 1 \end{bmatrix} \tag{C.33}$$

To finish, it is created the observability matrix and with it, it is possible to do the observability test.

$$O = \begin{bmatrix} 0 & -0.0463 & -2.73 \\ 1 & 0.0260 & -0.0002 \\ 0 & -0.0146 & 0 \end{bmatrix} \tag{C.34}$$

$$Rank(O) = 2 \tag{C.35}$$

The values of the equations 2.27 till C.35 were obtained with the MATLAB® code C.5 and are an example of values obtained for this model. The values of ten points of this model can be observable at C.1.

rank(O)	Days 1	2	3	4	5	6	7	8	9	10
Only Biomass	2	2	2	2	2	2	2	2	2	2
Only Ethanol	3	3	3	3	3	3	3	3	3	3
Only Substract	2	2	2	2	2	2	2	2	2	2
Biomass and Ethanol	3	3	3	3	3	3	3	3	3	3
Biomass and Substract	2	2	2	2	2	2	2	2	2	2
Ethanol and Substract	3	3	3	3	3	3	3	3	3	3
All	3	3	3	3	3	3	3	3	3	3

Table C.1: Table of observability tests

By the values represented in the table C.1 the system is observable along the trajectory, only if the Ethanol is measured as an output. So, if the Ethanol is an output, the system is considered globally observable. For all the other possibilities the system failed the observability test.

```
syms X P S miumax Ksx qmax Ksp Yxs Yps

f1 = miumax*(S/(Ksx+S))*X; % biomas
f2 = qmax*(S/(Ksp+S))*X; % ethanol
f3 = -(1/Yxs)*f1-(1/Yps)*f2; % substract

jA = jacobian([f1, f2, f3], [X, P, S]);

aX = 5.437;
bS = 1.936;

A= subs(jA,{miumax, Ksx, qmax, Ksp, Yxs, Yps, X, S},{0.3, 57.74, 2.756, 166.33,
    0.063, 0.58, aX, bS});

[V,D]=eig(A);
C=[1 0 0]; % X P S
Aprima=V^-1*A*V;
Cprima=C*V;

Ob=obsv(Aprima,Cprima);
```

```
rankOb= rank(Ob)
```

Listing C.1: An example of Observability Code

C.6 Observability Matrix Terms

$$CA = \begin{bmatrix} CA_{11} & CA_{12} & CA_{13} \\ -\frac{k_{SX}\ \mu_{max} K_X e^{-k_{XE}\ E} X}{(K_X+S)^2} & k_{SX}\mu_{max}\frac{S}{K_X+S}\ k_{XE}\ e^{-k_{XE}\ E} X & -k_{SX}\mu_{max}\frac{S}{K_X+S}\ e^{-k_{XE}\ E} \end{bmatrix} \tag{C.36}$$

$$CA_{11} = \frac{k_{CTR}\ K_X\ (\mu_{max}\ e^{-k_{XE}\ E})^2\ X}{(K_X+S)^3}\left(\frac{k_{SX}\ K_X\ X}{K_X+S} + (k_{XE}\ k_{EX} X - 1)S\right) \tag{C.37}$$

$$CA_{12} = \frac{k_{CTR}\ k_{XE}\ (\mu_{max}\ e^{-k_{XE}\ E})^2 X\ S}{(K_X+S)^2}\left(\frac{k_{SX}\ K_X\ X}{K_X+S} + (k_{XE}\ k_{EX}\ X - 1)S\right) \tag{C.38}$$

$$CA_{13} = \frac{k_{CTR}\ (\mu_{max} e^{-k_{XE}\ E})^2\ S}{(K_X+S)^2}\left(\frac{k_{SX}\ K_X X}{K_X+S} + (k_{XE}\ k_{EX}\ X - 1)S\right) \tag{C.39}$$

$$CA^2 = \begin{bmatrix} CA_{11}^2 & CA_{12}^2 & CA_{13}^2 \\ CA_{21}^2 & CA_{22}^2 & CA_{23}^2 \end{bmatrix} \tag{C.40}$$

$$CA_{11}^2 = \left(\frac{k_{SX}\ K_X\ X}{K_X+S} + (k_{XE}\ k_{EX} X - 1)S\right)\frac{k_{CTR}\ K_X (\mu_{max} e^{-k_{XE}\ E})^3\ X}{(K_X+S)^4} \left(\frac{k_{SX}\ K_X\ X}{K_X+S} - (k_{XE}\ k_{EX} X + 1)S\right) \tag{C.41}$$

$$CA_{12}^2 = \left(\frac{k_{SX}\ K_X X}{K_X+S} + (k_{XE}\ k_{EX}\ X - 1)S\right)\frac{k_{CTR}\ k_{XE}\ (\mu_{max} e^{-k_{XE}\ E})^3\ S\ X}{(K_X+S)^3} \left(\frac{k_{SX}\ K_X\ X}{K_X+S} - (k_{EX}\ k_{XE}\ X\ S + 1)S\right) \tag{C.42}$$

$$CA_{13}^2 = \left(\frac{k_{SX}\ K_X X}{K_X+S} + (k_{XE}\ k_{EX}\ X - 1)S\right)\frac{k_{CTR}\ (\mu_{max} e^{-k_{XE}\ E})^3\ S}{(K_X+S)^3} \left(\frac{k_{SX}\ K_X\ X}{K_X+S} - (k_{XE}\ k_{EX}\ X\ S + 1)S\right) \tag{C.43}$$

$$CA_{21}^2 = \frac{k_{SX}(\mu_{max} e^{-k_{XE}\ E} K_X\ X)^2}{(K_X+S)^3}\left(\frac{k_{SX}}{K_X+S} - (k_{EX}\ k_{XE}\ X - 1)S\right) \tag{C.44}$$

$$CA_{22}^2 = \frac{k_{XE}\ k_{SX}(\mu_{max} e^{-k_{XE}\ E})^2 X\ S}{(K_X+S)^2}\left(\frac{k_{SX}\ K_X\ X}{K_X+S} + (k_{EX} k_{XE}\ X\ - 1)S\right) \tag{C.45}$$

$$CA_{23}^2 = \frac{k_{SX}(\mu_{max} e^{-k_{XE}\ E})^2\ S}{(K_X+S)^2}\left(\frac{k_{SX}\ K_X\ X}{K_X+S} + (k_{EX} k_{XE}\ X\ - 1)S\right) \tag{C.46}$$

C.7 Reducing the Matrix into its Echelon Form

C.7.1 For S and CTR measurement:

$$O = \begin{bmatrix} O_{11} & O_{12} & O_{13} \\ 1 & 0 & 0 \\ O_{31} & O_{32} & O_{33} \\ O_{41} & O_{42} & O_{43} \\ O_{51} & O_{52} & O_{53} \\ O_{61} & O_{62} & O_{63} \end{bmatrix} \xrightarrow{L_1 \rightleftharpoons L_2} \begin{bmatrix} 1 & 0 & 0 \\ O_{11} & O_{12} & O_{13} \\ O_{31} & O_{32} & O_{33} \\ O_{41} & O_{42} & O_{43} \\ O_{51} & O_{52} & O_{53} \\ O_{61} & O_{62} & O_{63} \end{bmatrix} \begin{matrix} \\ \xrightarrow{L_2 = L_2 - O_{11} \times L_1} \\ \xrightarrow{L_3 = L_3 - O_{31} \times L_1} \\ \xrightarrow{L_4 = L_4 - O_{41} \times L_1} \\ \xrightarrow{L_5 = L_5 - O_{51} \times L_1} \\ \xrightarrow{L_6 = L_6 - O_{61} \times L_1} \end{matrix} \begin{bmatrix} 1 & 0 & 0 \\ 0 & O_{12} & O_{13} \\ 0 & O_{32} & O_{33} \\ 0 & O_{42} & O_{43} \\ 0 & O_{52} & O_{53} \\ 0 & O_{62} & O_{63} \end{bmatrix} \tag{C.47}$$

$$O = \begin{bmatrix} 1 & 0 & 0 \\ 0 & O_{12} & O_{13} \\ 0 & O_{32} & O_{33} \\ 0 & O_{42} & O_{43} \\ 0 & O_{52} & O_{53} \\ 0 & O_{62} & O_{63} \end{bmatrix} \xrightarrow{L_2 = \frac{L_2}{O_{12}}} \begin{bmatrix} 1 & 0 & 0 \\ 0 & 1 & \frac{O_{13}}{O_{12}} \\ 0 & O_{32} & O_{33} \\ 0 & O_{42} & O_{43} \\ 0 & O_{52} & O_{53} \\ 0 & O_{62} & O_{63} \end{bmatrix} \begin{matrix} \\ \\ \xrightarrow{L_3 = L_3 - O_{32} \times L_2} \\ \xrightarrow{L_4 = L_4 - O_{42} \times L_2} \\ \xrightarrow{L_5 = L_5 - O_{52} \times L_2} \\ \xrightarrow{L_6 = L_6 - O_{62} \times L_2} \end{matrix} \begin{bmatrix} 1 & 0 & 0 \\ 0 & 1 & \frac{O_{13}}{O_{12}} \\ 0 & 0 & O_{33} - O_{32} \times \frac{O_{13}}{O_{12}} \\ 0 & 0 & O_{43} - O_{42} \times \frac{O_{13}}{O_{12}} \\ 0 & 0 & O_{53} - O_{52} \times \frac{O_{13}}{O_{12}} \\ 0 & 0 & O_{63} - O_{62} \times \frac{O_{13}}{O_{12}} \end{bmatrix} \tag{C.48}$$

$$O = \begin{bmatrix} 1 & 0 & 0 \\ 0 & 1 & \frac{O_{13}}{O_{12}} \\ 0 & 0 & O_{33} - O_{32} \times \frac{O_{13}}{O_{12}} \\ 0 & 0 & O_{43} - O_{42} \times \frac{O_{13}}{O_{12}} \\ 0 & 0 & O_{53} - O_{52} \times \frac{O_{13}}{O_{12}} \\ 0 & 0 & O_{63} - O_{62} \times \frac{O_{13}}{O_{12}} \end{bmatrix} \begin{matrix} \\ \\ \xrightarrow{L_3 = \frac{L_3}{O_{33} - O_{32} \times \frac{O_{13}}{O_{12}}}} \\ \xrightarrow{L_4 = \frac{L_4}{O_{43} - O_{42} \times \frac{O_{13}}{O_{12}}}} \\ \xrightarrow{L_3 = \frac{L_5}{O_{53} - O_{52} \times \frac{O_{13}}{O_{12}}}} \\ \xrightarrow{L_3 = \frac{L_6}{O_{63} - O_{62} \times \frac{O_{13}}{O_{12}}}} \end{matrix} \begin{bmatrix} 1 & 0 & 0 \\ 0 & 1 & \frac{O_{13}}{O_{12}} \\ 0 & 0 & 1 \\ 0 & 0 & 1 \\ 0 & 0 & 1 \\ 0 & 0 & 1 \end{bmatrix} \begin{matrix} \\ \\ \\ \xrightarrow{L_4 = L_4 - L_3} \\ \xrightarrow{L_3 = L_5 - L_3} \\ \xrightarrow{L_3 = L_5 - L_3} \end{matrix} \begin{bmatrix} 1 & 0 & 0 \\ 0 & 1 & \frac{O_{13}}{O_{12}} \\ 0 & 0 & 1 \\ 0 & 0 & 0 \\ 0 & 0 & 0 \\ 0 & 0 & 0 \end{bmatrix} \tag{C.49}$$

C.7.2 For only CTR measurement:

$$O = \begin{bmatrix} O_{11} & O_{12} & O_{13} \\ O_{31} & O_{32} & O_{33} \\ O_{51} & O_{52} & O_{53} \end{bmatrix} \xrightarrow{L_1 = \frac{L_1}{O_{11}}} \begin{bmatrix} 1 & \frac{O_{12}}{O_{11}} & \frac{O_{13}}{O_{11}} \\ O_{31} & O_{32} & O_{33} \\ O_{51} & O_{52} & O_{53} \end{bmatrix} \tag{C.50}$$

$$O = \begin{bmatrix} 1 & \frac{O_{12}}{O_{11}} & \frac{O_{13}}{O_{11}} \\ O_{31} & O_{32} & O_{33} \\ O_{51} & O_{52} & O_{53} \end{bmatrix} \begin{matrix} \xrightarrow{L_2 = L_2 - O_{31} \times L_1} \\ \xrightarrow{L_3 = L_3 - O_{51} \times L_1} \end{matrix} \begin{bmatrix} 1 & \frac{O_{12}}{O_{11}} & \frac{O_{13}}{O_{11}} \\ 0 & O_{32} - O_{31}\frac{O_{12}}{O_{11}} & O_{33} - O_{31}\frac{O_{13}}{O_{11}} \\ 0 & O_{52} - O_{51}\frac{O_{12}}{O_{11}} & O_{53} - O_{51}\frac{O_{13}}{O_{11}} \end{bmatrix} \tag{C.51}$$

$$O = \begin{bmatrix} 1 & \frac{O_{12}}{O_{11}} & \frac{O_{13}}{O_{11}} \\ 0 & O_{32} - O_{31}\frac{O_{12}}{O_{11}} & O_{33} - O_{31}\frac{O_{13}}{O_{11}} \\ 0 & O_{52} - O_{51}\frac{O_{12}}{O_{11}} & O_{53} - O_{51}\frac{O_{13}}{O_{11}} \end{bmatrix} \xrightarrow{L_2 = \frac{L_2}{O_{32} - O_{31}\frac{O_{12}}{O_{11}}}} \begin{bmatrix} 1 & \frac{O_{12}}{O_{11}} & \frac{O_{13}}{O_{11}} \\ 0 & 1 & \frac{O_{33} - O_{31}\frac{O_{13}}{O_{11}}}{O_{32} - O_{31}\frac{O_{12}}{O_{11}}} \\ 0 & O_{52} - O_{51}\frac{O_{12}}{O_{11}} & O_{53} - O_{51}\frac{O_{13}}{O_{11}} \end{bmatrix} \tag{C.52}$$

$$\xrightarrow{L_3 = L_3 - (O_{52} - \frac{O_{51}O_{12}}{O_{11}}) \times L_2} \begin{bmatrix} 1 & \frac{O_{12}}{O_{11}} & \frac{O_{13}}{O_{11}} \\ 0 & 1 & \frac{O_{33} - O_{31}\frac{O_{13}}{O_{11}}}{O_{32} - O_{31}\frac{O_{12}}{O_{11}}} \\ 0 & 0 & O_{53} - O_{51}\frac{O_{13}}{O_{11}} - (O_{52} - \frac{O_{51}O_{12}}{O_{11}})(\frac{O_{33} - O_{31}\frac{O_{13}}{O_{11}}}{O_{32} - O_{31}\frac{O_{12}}{O_{11}}}) \end{bmatrix} \tag{C.53}$$

$$\xrightarrow{L_3 = \frac{L_3}{O_{53} - O_{51}\frac{O_{13}}{O_{11}} - (O_{52} - \frac{O_{51}O_{12}}{O_{11}})(\frac{O_{33} - O_{31}\frac{O_{13}}{O_{11}}}{O_{32} - O_{31}\frac{O_{12}}{O_{11}}})}} \begin{bmatrix} 1 & \frac{O_{12}}{O_{11}} & \frac{O_{13}}{O_{11}} \\ 0 & 1 & \frac{O_{33} - O_{31}\frac{O_{13}}{O_{11}}}{O_{32} - O_{31}\frac{O_{12}}{O_{11}}} \\ 0 & 0 & 1 \end{bmatrix} \tag{C.54}$$

Appendix D

Experimental and Laboratory information

D.1 Beer Measurements

D.1.1 Specific Gravity

C. J. Marchbancks in [40] defines Specific Gravity for beer as *"the density of beer or wort at standard temperature and pressure (20°C, 760 mm Hg) measured by saccharometer, hydrometer, or refractometer"*. The measurement of the wort specific gravity is important because it indicates the potential alcoholic strength of a beer.
Specific gravity is unitless since it is a ratio of the density of the beer/wort to the density of a given reference, mostly distilled water. In the literature, there are a few measurement scales that Brewers use to refer to the specific gravity and there are tables where there are comparison values between them. One of these tables can be seen in Annexe I. It is also possible to use formulas to convert the measurements like D.1, where SG is specific gravity, ${}^{o}P$ is degrees plato and ${}^{o}Bx$ is degrees Brix.

$$SG = \frac{{}^{o}P}{258.6 - \frac{{}^{o}P\ 227.1}{258.2}} = \frac{{}^{o}Bx}{258.6 - \frac{{}^{o}Bx\ 227.1}{258.2}} + 1 \tag{D.1}$$

D.1.1.1 Balling Scale

Devised by Karl Balling in 1843, the balling scale establishes a set of tables relating the weight percentage of sucrose to the specific gravity of the solution at a temperature of 17.5ºC. The Balling Scale, one of the oldest of its kind to remain, is a measurement of the dissolved sugar concentration in the wort, says [41]. Its measurements are expressed as ºBalling (degrees Balling or ºB) and this scale is gradually being replaced by the Plato and Brix scales due to its inaccuracy and the temperature constraints.

D.1.1.2 Plato Scale

Following [42], Plato scale also measures the concentration of the dissolved solids in the wort, like the Balling scale, but, the experiments were carried out at 20ºC. Its measurements are expressed in degrees plato (ºP) and is used to quantify the concentration of extract over a weight percentage. As an example, 10ºP of wort will contain 10g of extract per 100g of wort, says [42].
The literature [42] suggests that for every 1ºP generates 0.4% of alcohol by volume of beer approximately, depending on the concentration of different types of sugar and yeast behaviour. So, following this statement, a 10ºP wort will probably produce a beer of 4% of alcohol power if the sugars and yeast corroborates.

D.1.1.3 Brix Scale

The Brix scale is used to calculate an approximated potential alcohol content of a beer, as said by [43]. The reason why it is an approximation, is that the Brix scale expresses the number of grams of sucrose present in 100 grams of an aqueous solution, and wort/beer is a complex solution that has more chemical compounds than sucrose and water.
Valued from 1 to 100, the Brix scale measurements are expressed in degrees Brix (^{o}Bx) and for every 1 ^{o}Bx a 0.59% of potential of Alcohol by Volume (ABV) the beer will have. So, as an example, if ones wort measured 20 ^{o}Bx it has a approximately 11.8% ABV. Also following [43], this potential ABV conversion factor can range from 0.55 to 0.65, but the value 0.59 is the most used.

D.1.2 Temperature

Measured with a thermometer, temperature is considered one of the most important variables in a brewing process [44]. Because of its importance, temperature is widely studied. In the literature [1] [2] [28] [11], temperature is cited as a measured variable, as an operation variable, as an operational signal and as a state variable in mathematical models. Following [45], temperature has a strong relationship with the quality of the beer. So measuring it is of the utmost importance. R. Mosher in [37] affirms that mechanical thermostats are used for heaters, hot plates and fermenters that need heating rather than cooling. Thermocouples are also used, in more complex sets, where involves controllers, such as PIDs or with digital thermometers. These thermometers are calibrated following a reference in a scale. The International System of Units (SI) states Kelvin scale as the official scale for temperature, and its unit (K) as the standard international system unit for temperature, but Brewers commonly uses Fahrenheit scale (^{o}F) and Celsius scale (^{o}C) [44].

D.1.3 pH

Following [2], pH is a scale to determine how acidic/alkaline a water-based solution is. A solution is considered acidic if its pH is less than 7, 7 represents the neutral value and from 7 till 14 is alkaline. A beer, normally will range its pH value as 4.
In brewery processes is recommended to measure pH, specially in the mash, using a pH meter. There are different models in the market that can be applied, but all must be calibrated [46]. It is not recommended the used of litmus papers because this method is imprecise for beer brewing [47].
There is also the possibility to estimate the pH value of the mash theoretically. Some brewing softwares, like BeerSmith^{TM}, are capable of estimate the mash pH and determine adjustments of the water before starting brewing [48].

D.2 Equipment

D.2.1 Devices

D.2.1.1 Mashing process

§1 Brewing System
During the mashing and boiling process (1.1), which needs control of temperature, one used Grainfather G30 brewing system. This system is a four pieces brewing kit design for homebrewing from 4.5 to 9kg worth of grains and with a maximum volume capacity of 30L. The first piece is a stainless steel pan coupled with a PID control heating system, the second one is also a stainless steel expandable grain basket for the mashing, the third is the Bluetooth controller and the forth is the counter flow chiller.

The entire operation is controlled by this Bluetooth controller and the recipes, set points and manual operations can be done locally or through the Grainfathers application installed on a smartphone connected via Bluetooth to the controller or on its website.

D.2.1.2 Fermenting process

§1 Fermenter

In the course of the experiments it was seen the need of the change of the sizes of the fermenter due to time constraints and fermentation issues. It was used fermenters with volumes that varies from 5L till 20L. The choice of the fermenters were based on size, ways of manipulation, food adequacy, shape, material and color. At the end, there was three types of fermenters, and in theory, their shapes should not affect the quality of the beer.

§2 Airlock

Airlocks are devices that allows CO_2, released during the fermentation process, to escape the fermenter while preventing the air to enter the fermenter, and consequently avoiding the contamination and oxidation of the beer. It is also a security item since it avoids the pressurization of the fermenter.

There are two kinds of airlocks there are commonly used in the homebrewing. In this project, it was tested three kinds of airlocks that can be seen in the Figure D.1. The number one is the most used airlock in the market, the second one is the second most used airlock in the market and the one that will be implemented in the CO_2 sensor development due to its physical movement and the third one is the Speidels airlock used when tested the ultrasound CO_2 sensor version due to its size and color.

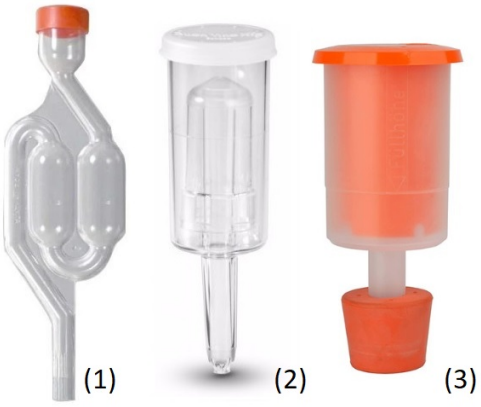

Figure D.1: Tested Airlocks

For these airlocks to work they must be half filled with a solution that can be water but preferably a sanitizing solution like alcohol. The sanitizing solution is used to prevent contamination into the beer in case the solution goes inside the fermenter.

Their mechanism is very simple. When the pressure inside the fermenter exceeds the atmospheric pressure, the gas will push its way out the vessel through the solution as bubbles into the outside air.

The movement

The airlock 2 of Figure D.1, which was used for most of this project, has a structure (fixed part, number 2 of Figure D.2) and a moving part (number 3 of Figure D.2).

Figure D.2: Airlock 3 pieces

When these airlocks are in operation, and the pressure inside the fermenter starts to get higher than the atmospheric one, the moving part start its movement up. When the pressure hits a boundary condition, there is the creation of a bubble/blop inside the solution of the airlock. After that, the moving part goes down. Figure D.3 shows the movement over time of the airlock moving part pointing out the moment where a bubble comes out of the airlock.

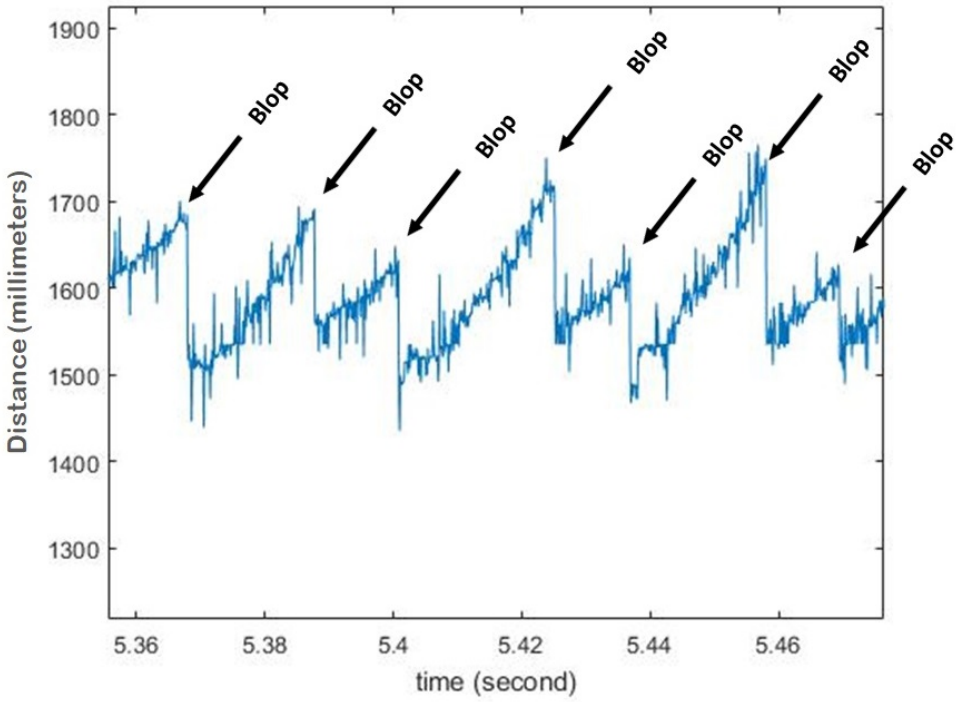

Figure D.3: CO_2 Blops

D.2.2 Instrumentation

D.2.2.1 Auxiliary Devices

To assist the mashing process and cooling (after the boiling process) it was used two measurement devices. A thermometer was used to measure the cooled temperature of the wort coming out of the counter flow chiller and the temperature of the water used to hydrate the yeast. The laboratory made available a portable thermocouple for this project. The other measurement device that assisted was a digital pH meter PH-107 that was used only during the mashing to observe the pH of the solution.

D.2.2.2 Tilt™ Hydrometer

Tilt™, a hydrometer and a thermometer, is an evasive device designed for home brewing, that provides the Brewer instantly reads of both signals on their smartphones through a Bluetooth® 4.0+ communication. The Tilt™ comes calibrated with a specific gravity accuracy of ± 0.002 in a range of 0.990 to 1.120 and a temperature accuracy of ± 0.5 ^oC.
This device has its own App which is compatible with the main smartphones and has even its own acquisition device. In its App, it offers a way to save your data in a Google Sheets template. And different from the above device, this one is inserted inside of the fermenter, so, if it was proper sanitized, it avoids contamination and waists due to specific gravity sampling.

§1 Data Format
Tilt™ Hydrometer transmits data via iBeacon protocol. It sends two messages, where the first doesn't have any sensor data and the second contains the temperature and specific gravity values. The data package is a 336 bits divided in 42 hexadecimal numbers. An example of a data package can be seen in Figure D.4 and in Annex III there is an explanation list of all this numbers found in [49].

```
> 04 3E 27 02 01 00 00 5A 09 9B 16 A3 04 1B 1A FF 4C 00 02 15
  A4 95 BB 10 C5 B1 4B 44 B5 12 13 70 F0 2D 74 DE 00 44 03 F8
  C5 C7
```

Figure D.4: Data Format of Tilt™ Hydrometer D.4.

The values of temperature and specific gravity can be found right after the device Universally Unique Identifier (UUID), signed in blue (Specific Gravity) and red (Temperature) in Figure D.4. Both are 16 bit unsigned integers with their most significant bits being the first ones. The temperature is given in degrees Fahrenheit while specific gravity is multiplied by 1000. So, for the Figure D.4 temperature will be 68^{o}F with a specific gravity of 1.016.

D.2.3 Softwares

Softwares are used throughout this project, from the recipe planning to handling the acquired data, they've been used extensively. In the experimental part, softwares were used for the brewing process (Grainfather), the acquisition of data (ARDUINO® IDE) and treatment of data (Excel, PLX-DAQ, MATLAB ®).

D.2.3.1 Grainfather Community

To operate the Grainfather G30 brewing system, as explained in the Subsection §1, the operator can use an application installed in its smartphone or remotely. This application is logged to an account to link to a community and a website of the company.
In this website it is possible to find recipes, keep a track in your brews, check equipments, check profiles, ask for help, read articles and more. In it, one added all the recipes for the experimental parts and the equipment profile. It is important to say that in the website it is not possible to operate the brewing system, for safety reasons.
The application, after connected via Bluetooth to the Grainfather G30 brewing system is capable of controlling and monitoring the system in a range. It will follow one of the recipes implemented in the website, and chosen by the operator. It can turn on/of the heater and the actuator motor, which are the only two actuators of the system, and it can skip, stop and add time to the steps of the recipe.

D.2.3.2 Data Acquisition and Treatment

The software used to program the microcontrollers for the acquisition of the data was the ARDUINO® IDE. It is an open-source program, with a big community and it was chosen due to the familiarity with the developer.
After the acquisition of the data the values measured by the sensors are wrapped by the microcontrollers and send to their serial port. Than, an acquisition software called Parallax Data Acquisition tool (PLX-DAQ) send this data directly into a Microsoft excel file. This tool also unwrap the data, and add receiving time. To finish, this data passes through a treatment in MATLAB® and if needed, it is counted the blops.

Annex I

Table of Brix, Plato and Specific Gravity Conversion

Brix	Plato	SG	Brix	Plato	SG	Brix	Plato	SG
0.0	0.0000	1.0000	13.4	13.4027	1.0543	26.8	26.7948	1.1140
0.2	0.1970	1.0008	13.6	13.6028	1.0551	27.0	26.9944	1.1150
0.4	0.3970	1.0016	13.8	13.8029	1.0560	27.2	27.1940	1.1159
0.6	0.5970	1.0024	14.0	14.0030	1.0568	27.4	27.3936	1.1168
0.8	0.7970	1.0031	14.2	14.2030	1.0577	27.6	27.5932	1.1178
1.0	0.9970	1.0039	14.4	14.4031	1.0586	27.8	27.7928	1.1187
1.2	1.1970	1.0047	14.6	14.6031	1.0594	28.0	27.9924	1.1197
1.4	1.3971	1.0054	14.8	14.8032	1.0603	28.2	28.1919	1.1206
1.6	1.5971	1.0062	15.0	15.0032	1.0611	28.4	28.3915	1.1216
1.8	1.7971	1.0070	15.2	15.2033	1.0620	28.6	28.5910	1.1225
2.0	1.9972	1.0078	15.4	15.4033	1.0628	28.8	28.7905	1.1235
2.2	2.1972	1.0086	15.6	15.6033	1.0637	29.0	28.9901	1.1244
2.4	2.3973	1.0094	15.8	15.8034	1.0646	29.2	29.1896	1.1254
2.6	2.5973	1.0101	16.0	16.0034	1.0654	29.4	29.3891	1.1263
2.8	2.7974	1.0109	16.2	16.2034	1.0663	29.6	29.5886	1.1273
3.0	2.9975	1.0117	16.4	16.4034	1.0672	29.8	29.7880	1.1282
3.2	3.1975	1.0125	16.6	16.6034	1.0680	30.0	29.9875	1.1292
3.4	3.3976	1.0133	16.8	16.8034	1.0689	30.2	30.1870	1.1302
3.6	3.5977	1.0141	17.0	17.0034	1.0698	30.4	30.3864	1.1311
3.8	3.7977	1.0149	17.2	17.2034	1.0706	30.6	30.5859	1.1321
4.0	3.9978	1.0157	17.4	17.4034	1.0715	30.8	30.7853	1.1330
4.2	4.1979	1.0165	17.6	17.6034	1.0724	31.0	30.9847	1.1340
4.4	4.3980	1.0173	17.8	17.8034	1.0733	31.2	31.1841	1.1350
4.6	4.5981	1.0181	18.0	18.0033	1.0741	31.4	31.3835	1.1359
4.8	4.7982	1.0189	18.2	18.2033	1.0750	31.6	31.5829	1.1369
5.0	4.9983	1.0197	18.4	18.4033	1.0759	31.8	31.7823	1.1379
5.2	5.1984	1.0205	18.6	18.6032	1.0768	32.0	31.9817	1.1389
5.4	5.3985	1.0213	18.8	18.8032	1.0777	32.2	32.1810	1.1398
5.6	5.5986	1.0221	19.0	19.0031	1.0785	32.4	32.3804	1.1408
5.8	5.7987	1.0229	19.2	19.2030	1.0794	32.6	32.5797	1.1418
6.0	5.9988	1.0237	19.4	19.4030	1.0803	32.8	32.7791	1.1428
6.2	6.1989	1.0245	19.6	19.6029	1.0812	33.0	32.9784	1.1437
6.4	6.3990	1.0253	19.8	19.8028	1.0821	33.2	33.1777	1.1447
6.6	6.5991	1.0261	20.0	20.0027	1.0830	33.4	33.3770	1.1457
6.8	6.7992	1.0269	20.2	20.2026	1.0839	33.6	33.5763	1.1467
7.0	6.9994	1.0277	20.4	20.4025	1.0848	33.8	33.7756	1.1477
7.2	7.1995	1.0285	20.6	20.6024	1.0857	34.0	33.9749	1.1487
7.4	7.3996	1.0294	20.8	20.8023	1.0866	34.2	34.1741	1.1497
7.6	7.5997	1.0302	21.0	21.0021	1.0875	34.4	34.3734	1.1507
7.8	7.7998	1.0310	21.2	21.2020	1.0884	34.6	34.5727	1.1516
8.0	7.9999	1.0318	21.4	21.4018	1.0892	34.8	34.7719	1.1526
8.2	8.2000	1.0326	21.6	21.6017	1.0901	35.0	34.9711	1.1536
8.4	8.4002	1.0334	21.8	21.8015	1.0911	35.2	35.1703	1.1546
8.6	8.6003	1.0343	22.0	22.0014	1.0920	35.4	35.3695	1.1556
8.8	8.8004	1.0351	22.2	22.2012	1.0929	35.6	35.5687	1.1566
9.0	9.0005	1.0359	22.4	22.4010	1.0938	35.8	35.7679	1.1576
9.2	9.2006	1.0367	22.6	22.6008	1.0947	36.0	35.9671	1.1586

Figure I.1: Part 1 of 2 of Table of Brix, Plato and Specific Gravity Conversion [50]

9.4	9.4007	1.0376
9.6	9.6009	1.0384
9.8	9.801	1.0392
10.0	10.0011	1.0400
10.2	10.2012	1.0409
10.4	10.4013	1.0417
10.6	10.6014	1.0425
10.8	10.8015	1.0434
11.0	11.0016	1.0442
11.2	11.2017	1.0450
11.4	11.4018	1.0459
11.6	11.6019	1.0467
11.8	11.8020	1.0475
12.0	12.0021	1.0484
12.2	12.2022	1.0492
12.4	12.4023	1.0501
12.6	12.6024	1.0509
12.8	12.8025	1.0518
13.0	13.0026	1.0526
13.2	13.2027	1.0534

22.8	22.8006	1.0956
23.0	23.0004	1.0965
23.2	23.2002	1.0974
23.4	23.4000	1.0983
23.6	23.5997	1.0992
23.8	23.7995	1.1001
24.0	23.9992	1.1011
24.2	24.1990	1.1020
24.4	24.3987	1.1029
24.6	24.5984	1.1038
24.8	24.7982	1.1047
25.0	24.9979	1.1057
25.2	25.1976	1.1066
25.4	25.3972	1.1075
25.6	25.5969	1.1084
25.8	25.7966	1.1094
26.0	25.9963	1.1103
26.2	26.1959	1.1112
26.4	26.3956	1.1122
26.6	26.5952	1.1131

36.2	36.1663	1.1596
36.4	36.3655	1.1606
36.6	36.5646	1.1617
36.8	36.7638	1.1627
37.0	36.9629	1.1637
37.2	37.1620	1.1647
37.4	37.3612	1.1657
37.6	37.5603	1.1667
37.8	37.7594	1.1677
38.0	37.9585	1.1688
38.2	38.1576	1.1698
38.4	38.3566	1.1708
38.6	38.5557	1.1718
38.8	38.7548	1.1728
39.0	38.9538	1.1739
39.2	39.1529	1.1749
39.4	39.3519	1.1759
39.6	39.5509	1.1770
39.8	39.7500	1.1780
40.0	39.9490	1.1790

Figure I.2: Part 2 of 2 of Table of Brix, Plato and Specific Gravity Conversion [50]

Annex II

Parameters for beer fermentation

Kinetic parameters for beer fermentation with free and immobilized cells - *ORIGINAL EXTRACT – 13%*

Model parameters								*Efficiency coefficients*		*Model error*
μ_{max}	K_{sx}	q_{pmax}	K_{sp}	$Y_{x/s}$	$Y_{p/s}$	K_{ix}	K_{ip}	η_u	η_q	
d^{-1}	$g.dm^{-3}$	$g.(g.d)^{-1}$	$g.dm^{-3}$	-	-	$g.dm^{-3}$	$g.dm^{-3}$	-	-	
Saccharomyces cerevisiae S-23 (bottom fermented yeasts)										
Monod										
Free cells										
0.224	*150*	*3.839*	*150*	*0.124*	*0.476*	-	-	*1.339*	*1.259*	*0.133*
Immobilized cells										
0.300	*221.420*	*4.901*	*226.72*	*0.063*	*0.58*	-	-			*0.370*
Tiessier										
Free cells										
0.27	*200*	*2.437*	*100*	*0.0621*	*0.6*	-	-	*1.092*	*1.02*	*0.691*
Immobilized cells										
0.294	*175*	*2.485*	*12.3*	*0.0527*	*0.7*	-	-			
Aiba										
Free cells										
0.527	*200*	*6.665*	*200*	*0.125*	*0.512*	*0.0361*	*0.023*	*0.620*	*0.823*	*0.143*
Immobilized cells										
0.327	*200*	*5.487*	*200*	*0.081*	*0.56*	*0.009*	*0.009*			*0.102*
Saccharomyces cerevisiae S-33 (top fermented yeasts)										
Monod										
Free cells										
0.395	*221.214*	*3.297*	*200*	*0.065*	*0.61*	-	-	*0.559*	*0.879*	*0.192*
Immobilized cells										
0.221	*158.11*	*2.897*	*200*	*0.071*	*0.59*	-	-			*0.210*
Tiessier										
Free cells										
0.307	*178.12*	*2.995*	*150*	*0.074*	*0.597*	-	-	*0.597*	*0.708*	*0.347*
Immobilized cells										
0.221	*199.78*	*2.123*	*150*	*0.075*	*0.61*	-	-			*0.214*
Aiba										
Free cells										
0.321	*124.21*	*6.130*	*100*	*0.054*	*0.59*	*0.123*	*0.065*	*2.038*	*0.747*	*0.464*
Immobilized cells										
0.654	*200*	*4.578*	*164*	*0.101*	*0.55*	*0.094*	*0.009*			*0.214*

Figure II.1: Kinetic parameters for beer fermentation with free and immobilized cells - ORIGINAL EXTRACT - 13% [10]

Annex III

Explanation of Tilt^{TM} data

04: HCI Packet Type HCI Event
3E: LE Meta event
27: Parameter total length (39 octets)
02: LE Advertising report sub-event
01: Number of reports (1)
00: Event type connectable and scannable undirected advertising
00: Public address type
5A: address
09: address
9B: address
16: address
A3: address
04: address
1B: length of data field (27 octets)
1A: length of first advertising data (AD) structure (26)
FF: type of first AD structure - manufacturer specific data
4C: manufacturer ID - Apple iBeacon
00: manufacturer ID - Apple iBeacon
02: type (constant, defined by iBeacon spec)
15: length (constant, defined by iBeacon spec)
A4: device UUID
95: device UUID
BB: device UUID
10: device UUID
C5: device UUID
B1: device UUID
4B: device UUID
44: device UUID
B5: device UUID
12: device UUID
13: device UUID
70: device UUID
F0: device UUID
2D: device UUID
74: device UUID
DE: device UUID
00: major - temperature (in degrees fahrenheit)
44: major - temperature (in degress fahrenheit)

03: minor - specific gravity (x1000)
F8: minor - specific gravity (x1000)
C5: TX power in dBm
C7: RSSI in dBm