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Bachelor Thesis

Modelling the Ethanol production by *Saccharomyces cerevisiae* while Air Limitation

by

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Declaration

I hereby declare and confirm that the master thesis “Optimal Control of a Cell culture” is entirely the result of my own work except where otherwise indicated.

Abstract

Saccharomyces cerevisiae is a type of yeast that has been used since many years ago to winemaking, baking and brewering. In the industrial process of producing *Saccharomyces cerevisiae*, called baker's yeast, there is an undesired production of ethanol also under aerobic conditions which is called the Crabtree effect. During the growth of cells ethanol can also be produced under anaerobic conditions caused by the air limitation. The aim of this thesis is to create a model that is able to simulate ethanol production caused by the air limitation during the growth of a culture in a fed-batch reactor. To formulate the model, the respiration of *Saccharomyces cerevisiae* and the physical-principles of the mass transfer in a fed-batch reactor were analysed. The model is tested in different situations and with different parameters. To reduce the amount of ethanol and to increase the biomass production the glucose feed rate that is fed to the reactor was optimized.

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Chapter 1

Introduction

The growth of *Saccharomyces cerevisiae* is a process used extensively in many industries, such as the production of food, alcoholic beverages, pharmaceuticals, fine chemicals and enzymes. The yeast cells are produced in a series of fed-batch reactors. During the production, cells growth consuming oxygen and glucose which are fed to the reactor. During the process there is an undesired production of ethanol due to two effects, the Crabtree effect and the anerobic respiration. When the concentration of ethanol is higher than a certain value, industries can not sol the product. Therefore, it is necessary to develop a dynamic model that helps to avoid both undesired production of ethanol by the cells.

In chapter 1, the biology of *Saccharomyces cerevisiae* is explained. Chapter 2 describes the methods to model the growth of *Saccharomyces cerevisiae* in a fed-batch reactor. In the chapter 3, it is presented the dynamic model and the results of the simulation and optimization.

1.1 *Saccharomyces cerevisiae*

Saccharomyces cerevisiae is an eukaryote cell. It is sorted inside the unicellular fungus whose life cycle can be haploid or diploid.

Saccharomyces cerivisiae is a key organism to study biological issues. That is because it is ease of cultivation. Moreover, this type of cell stands out because it has a simple DNA trans-

formation system and can be manipulated in the laboratory with the minimum precautions because it is not a pathogenic organism.

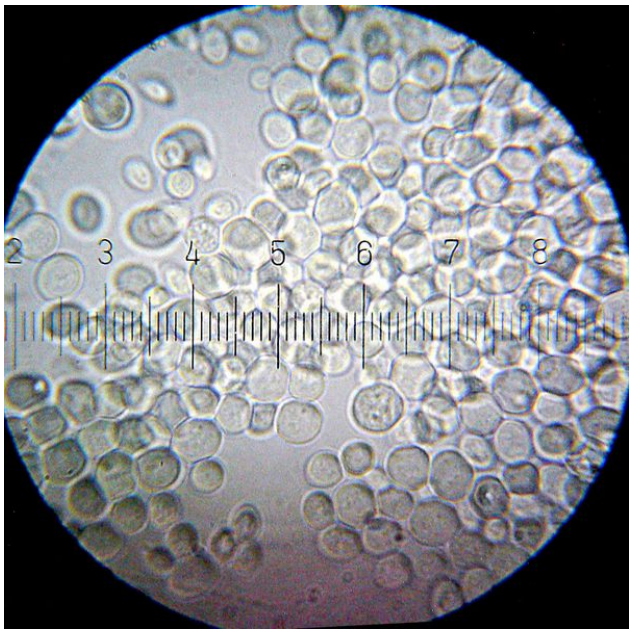


Figure 1.1: *Saccharomyces cerevisiae*[1].

Figure 1.1 shows cells shape of this kind of yeast trough a microscopy.

In addiction to its applications at the laboratory level, it is a key micro-organism in the in some industries due to its fast growth and its ability to produce ethanol in the fermentation [2].

1.1.1 Fungi

Saccharomyces cerevisiae is a type of cell which belongs to the kingdom fungi. The kingdom Fungi includes eukaryotic, multicellular organisms.

Based on their lifestyle, fungi are described by the following characteristics [3].

1. Nutrition.

   The nutrition of fungus is based on the absorption of organic nutrients. The absorption takes place through a process of osmotic absorption.

2. Vegetative state.

   The vegetative state is composed by filaments called hyphae which can be composed by one or more cells. The group of all hyphaes form the mycelium which absorbs the mineral compounds to feed fungus.

3. Cell wall.

   All fungus have a cell wall composed by chitin which is a white substance composed by the union of varies molecules of a nitrogenous sugar called N-acetylglucosamine.

4. Reproduction.

   The reproduction of fungus takes place through the creation of spores. Spores can support long time until ambient conditions are favourable for the germination. Fungus can create sexual spores through the meiosis and/or asexual spores through the mitosis.

5. Habitat.

   The habitat of fungus use to be warm and wet habitats where there are plenty of organic compounds.

6. Ecology.

   They can be saprotrophs if they consume remains of alive organisms, parasites if they consume the organica material of the alive being where they live, symbionts if they are associated with plants where they live

7. Variety.

   About 80 000 to 120 000 species of Fungus have been discovered. However, the total number of fungus has been estimated to be higher than 1.5 million of species.

   Cosmopolitan.

Fungi can be, on the basis of their structure, divided into yeasts, molds and mushrooms [4].

1.1.2 Respiration of *Saccharomyces cerevisiae*

Cellular respiration is the set of biochemical reaction that take place inside cells. Figure 1.2 shows the different processes involved in the cellular respiration.

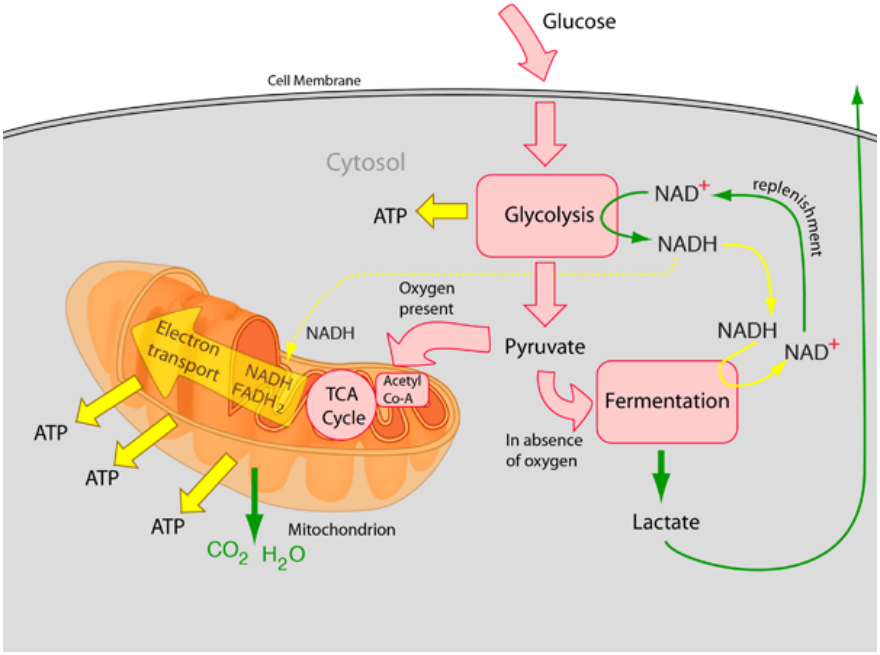


Figure 1.2: Cellular respiration [5].

Through respiration, cells degrade organic compounds to obtain energy for the essential process of life. The main source of energy for cells is ATP so respiration can be understands as a production process of ATP. The cells respiration can be an aerobic respiration in the presence of oxygen or anaerobic.

Glycolysis

Glycolysis is a group of chemical reactions that fullfill the first step in the respiration of *Saccharomyces cerevisiae*. During glycolysis larger molecules of glucose are broken down to form pyruvate. Due to larger molecules are broken down to smaller ones, the process produce two molecules of ATP [5]. Glycolysis is shown in figure 1.3.

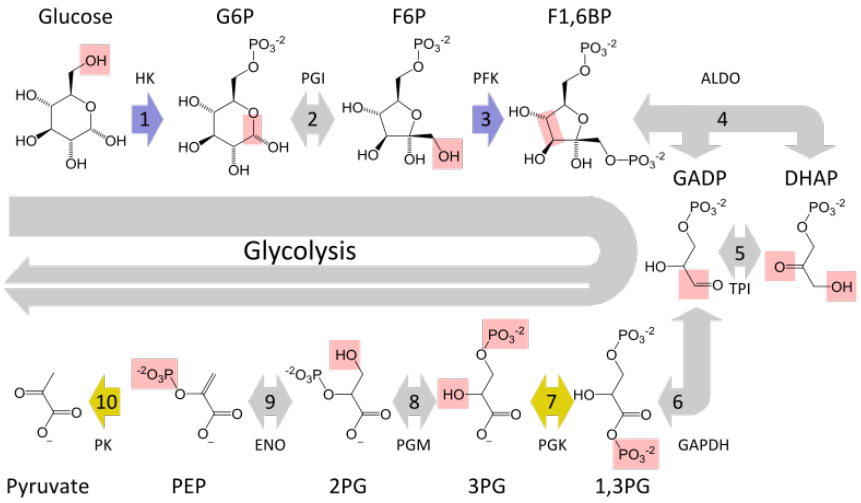


Figure 1.3: Glycolysis [6].

During the process a phosphate group is transferred from ATP to glucose, producing glucose-6-phosphate. Glucose-6-phosphate is converted into its isomer, fructose-6-phosphate. Later, a phosphate group is transferred from ATP to fructose-6-phosphate, producing fructose-1,6-bisphosphate. Fructose-1,6-bisphosphate splits to form dihydroxyacetone phosphate (DHAP) and glyceraldehyde-3-phosphate. Only glyceraldehyde-3-phosphate can continue through the next steps of glycolysis so DHAP is converted. After this step, Glyceraldehyde-3-phosphate loses two electrons and two protons forming 1,3-bisphosphoglycerate. 1,3-bisphosphoglycerate donates one of its phosphate groups converting into 3-phosphoglycerate which then is turned into its isomer. 2-phosphoglycerate loses a molecule of water and donates its phosphate group producing pyruvate [7]. The overall reaction of glycolysis is

$$C_6H_{12} + O_6 + 2\ ADP + 2\ NAD^+ + 2\ Pi \rightarrow 2\ C_3H_4O_3 + 2\ ATP + 2\ NADH + 2\ H_2O + 2\ H^+. \tag{1.1}$$

TCA cycle

If oxygen is avaliable, pyruvate is used as a substrate in the tricarboxylic acid cycle (TCA) which is shown in Figure 1.4. This cycle is a series of enzymatic reactions where acetyl CoA, formed from pyruvate, is completely oxidized into CO_2.

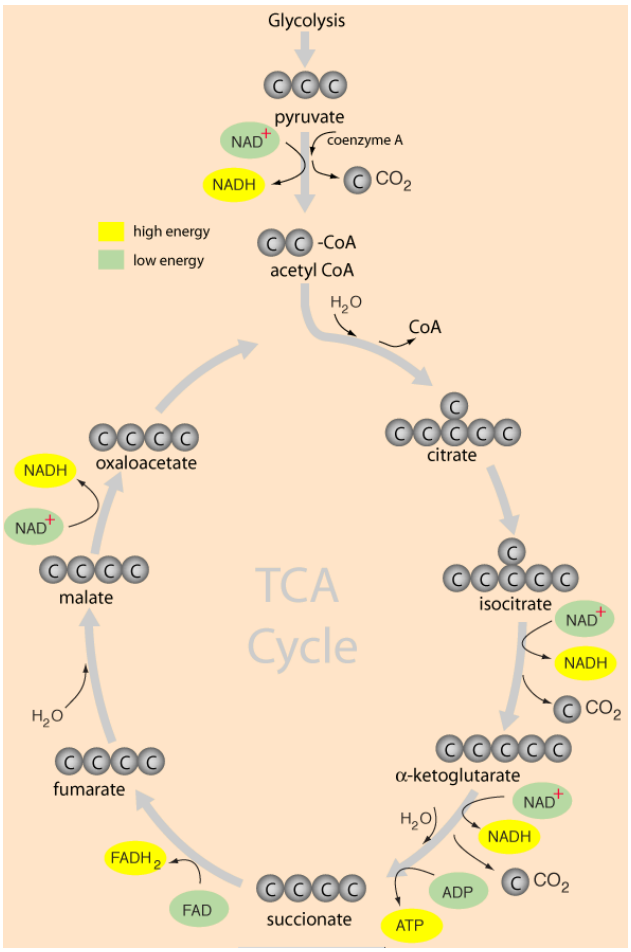


Figure 1.4: TCA cycle [5].

During the process, oxaloacetate transfers one acetyl group to acetyl CoA producing citrate. Citrate liberate two of its carbons forming two molecules of CO_2. 2 molecules of NAD^+, which are reduced to NADH, are used to carry out these reactions. The resulting four-carbon molecule is used them to produce one molecule of ATP, to reduce one molecule of FAD to $FADH_2$ and to reduce one molecule of NAD^+ to NADH. The final product is oxaloacetate which closes the cycle. The overall reaction is

$$C_3H_4O_3 + HCO_3^- + ADP \rightarrow Oxaloaceticacid + ATP + Pi. \tag{1.2}$$

After the TCA cycle, NADH and $FADH_2$ are in a high energy level. This energy is used inside the membrane of the mitochondria cells to synthesise ATP through a series of five biochemical reactions as it is shown in figure 1.5.

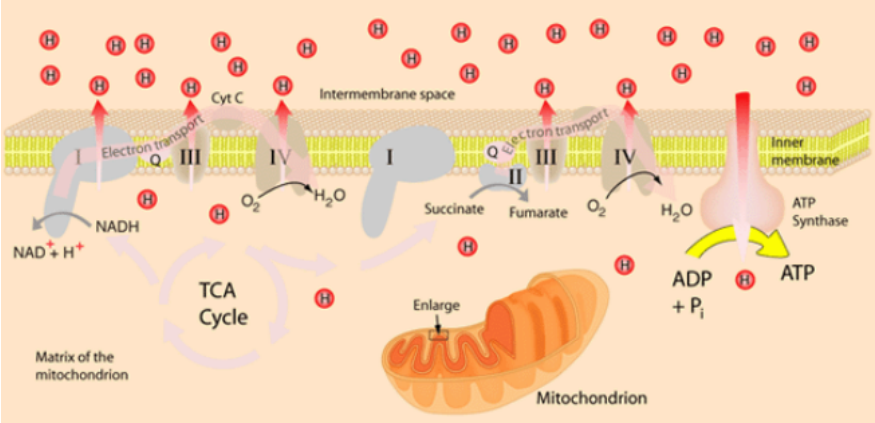


Figure 1.5: Electron transport chain [5].

The electron transport chain is composed of 4 complexes. NADH and $FADH_2$ are oxidized by these complexes and the resulting electrons are transferred to other electron acceptor which are in a lower energy level. The resulting energy of the electron transfer is used to pump H^+ ions out of the matrix of the mitochondrion. These ions create an energy gradient between the matrix of the mitochondrion and the intermembrane space. Due to the need of positive electrons to go back to the matrix because of the energy gradient, hydrogen ions pass through a protein complex called ATP synthase producing ATP from ADP.

Pentose phosphate pathway

During glycolysis, glucose is phosphorylated to produce glucose-6-phosphate. Pentose phosphate pathway is an important metabolic pathway where glucose-6-phophate can be converted to fructose-6-phosphate following glycolysis process or can follow the steps of the pentose phosphate pathway. This pathway can be divided into an oxidative and non-oxidative phase as is described in figure 1.6.

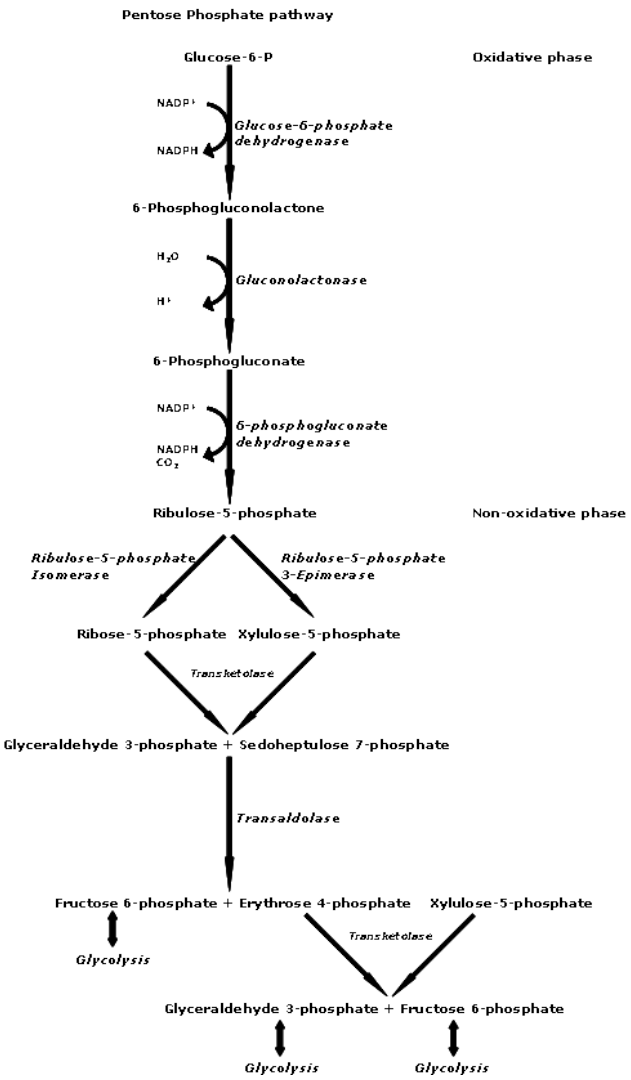


Figure 1.6: Pentose phosphate pathway [8].

During the oxidative phase ribose-5-phosphate is produced and $NADP^+$ is used as an oxidizing agent while NADPH is formed

$$\begin{aligned} &Glucose - 6 - phosphate + 2\ NADP^+ + H_2O \rightarrow Ribose - 5 - phosphate \\ &+ 2\ NADPH + 2\ H^+ + CO_2. \end{aligned} \tag{1.3}$$

In the non-oxidtive phase, ribose-5-phosphate reacts to fructose-6-phosphate and glyceraldehyde which is also used in glycolysis

$$\begin{aligned} &Ribose - 5 - phosphate \rightarrow 2\ Fructose - 6 - phosphate \\ &+ Glyceraldehyde - 3 - phosphate. \end{aligned} \tag{1.4}$$

The aims of the pentose phosphate pathway is to produce NADPH, which is used in biochemical reduction reactions, and to produce ribose-5-phophate which is used to build nucleotides such as deoxyribonucleic acid (DNA) and ribonucleic acid (RNA).

Depending on the needs of cells, ribose-5-phosphate can be used to build nucleotides or instead, can be used in the non-oxidative phase.

Synthesis and degradation of ethanol

If there is no oxygen available pyruvate is used to produce ethanol in two steps. Firstly, pyruvate is used to form acetaldehyde.

$$C_3H_4O_3 \rightarrow C_2H_4O + CO_2. \tag{1.5}$$

Then, acetaldehyde is reduced to ethanol.

$$C_2H_4O + 2\ NADH \rightarrow C_2H_6O + 2NAD^+. \tag{1.6}$$

Ethanol can be used as well as substrate that reacts to acetly-CoA which is an intermediate of the TCA cycle.

1.1.3 Application of *Saccharomyces cerevisiae*

The main application of *Saccharomyces cerevisiae* is found in the alimentary industry due to its capacity to form ethanol and CO_2 during the fermentation. The ethanol production is used in the industry to make some popular beverages while CO_2 has an important role in the pruduction of bread.

1. Ethanol production. Beer an wine are ones of the oldest drink known. The production of both are carried out by the anaerobic respiration of *Saccharomyces cerevisiae*.

   In the production of wine, the natural yeasts that are presented in the skin of grapes use the juice created when grapes are squashed as a substrate to produce ethanol in the fermentation. Nowadays, winemakers add yeast cultures depending on the desired type of wine at the end of the process.

In the production of beer, malt is used as carbon source in the fermentation. After the fermentation, the starches that composed malt are broken down in more simple molecules that can be metabolised by yeasts. After this process beer wort is mixed with *Saccharomyces cerevisiae* producing ethanol. When fermentation is carried out with warm temperatures Ale beer is created. If the fermentation takes place at cold temperatures, Lager beer is produced.

2. Bread production. During the preparation of bread, dried yeast is added with the rest of the ingredients such as flour, water or salt. Yeast feeds with the carbon sources that are presented in the flour producing ethanol and carbon dioxyde. The release of this gas brings about an increase in the bread volume. Ethanol is evaporated in the bread baking time.

Apart from the production of bread and alcoholic drink, yeast can be used as a nutritional supplement due to its content of B vitamins, minerals and proteins [1].

1.2 Production of yeast

Nowadays, yeast production is one of the most important process in the alimentary industry and more than 0.4 million of metric tonnes of yeast are made worldwide. An efficient production proces has been designed to accomplish with the yeast demand. This process is made up of 4 stages as it is described in figure 1.7.

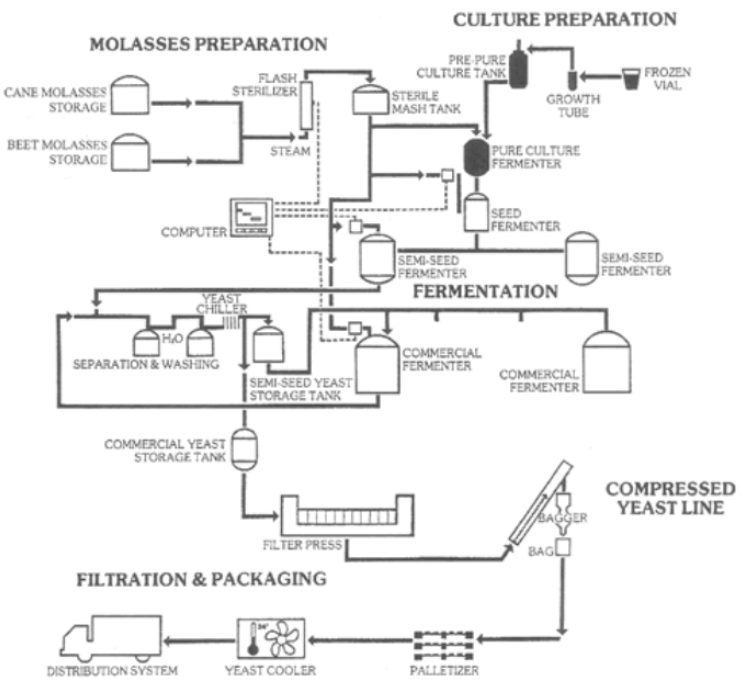


Figure 1.7: Yest production process [9].

Firstly the source of carbon must be prepared. Beet and cane used to be the raw material. Moreover, yeast also required certain mineral, vitamins and salt to grow as well as nitrogen, supplied as ammonia, and phosphate, supplied as phophoric acid. The energy source for yeast growth are sugars. Beet and cane molasses are commonly used as raw material which are stored in a separate tank. In addition to sugar, yeast also require certain minerals, vitamins and salts for growth. Some of these nutrients can be added to the sugar storage tank while others are added during the fermentation process. Both carbon sources must be sterilised to avoid the growth of some undesired micro-organism in the fermentation. The sterilised flow can be used then to fed the appropriate fermentation vessel [9].

Yeast production starts with a pure culture of *Saccharomyces cerevisiae* that is inoculated in a small pressure vessel. The product of this vessel is used to feed a multi-stage process where biomass grows from small vessels to larger fermenters. The multi-stage step is shown in figure 1.8

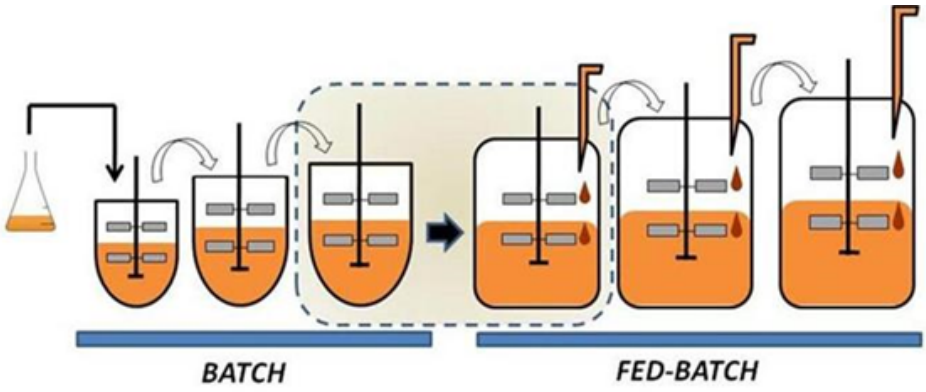


Figure 1.8: Multistage process[10].

In the initial batch phase, cells are exposed to the high sugars concentration and a constant aeration rate. Before the process has started,the pH value is adjusted while temperature aeration can be controlled during the fermentation [10].

Then, the culture is transferred to the fed-batch stage. Difference from the batch stage, the reactor is continuously fed with molasses while the biomass is growing as is presented in 1.9.

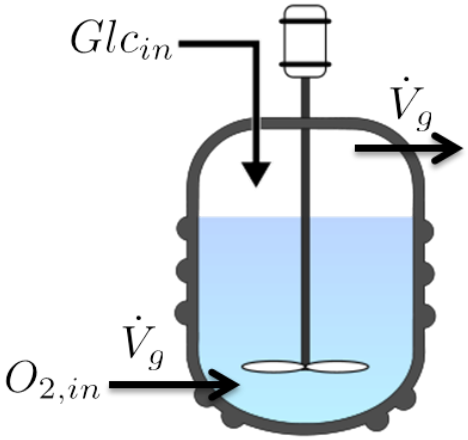


Figure 1.9: Fed-batch stage [10].

After the fermentation process, the broth has to pass through a separation stage to increase the concentration of solids. This stage is made up of nozzle centrifuges. After the centrifugation, yeast can be directly cooled, store and sold or can be pumped to a filter separation process where yeast are concentrated. The resulting solid is packed cooled in a refrigerator and delivered to the different customers.

1.2.1 Undesired production of ethanol

One problem during the production process is that the cell culture can switch to the undesired production of ethanol, and when the ethanol concentration is at the end of process greater than a tolerable range then the batch cannot be sold.

During the process, ethanol can be produced via the Crabtree effect or via the anaerobic respiration. When the sugar concentration is very high or the specific growth rate in aerobic cultures exceeds a critical value, ethanol is produced.

In a first growth phase, biomass is formed and ethanol is accumulated. In the following growth phase the produced ethanol is used as substrate for further growth [11]. The Crabtree effect can be explained on the molecular level by a repression/depression mechanism of respiration.

On the other hand, at the end of the process when the oxygen consumption by the cells is limited by low concentrations of dissolved oxygen, the production of ethanol is observed.

To show the tendency of these effects figure 1.10 shows an example of the Crabtree effect.

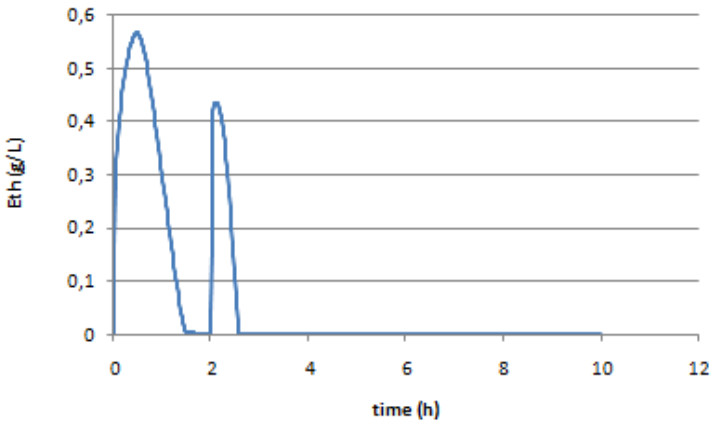


Figure 1.10: Ethanol production during yeast growth.

The aim of this thesis is to build a model which enables to simulate the last part of the process where the production of ethanol is due to air limitation.

Chapter 2

Methods

During the last years, mathematical modelling has been developed with the aim of expressing observed phenomenons through mathematical equations. One of the keys of this development has been the improvement in computing. Mathematical modelling and computing improvement has been very importan in chemical engineering where physical an chemical laws are complicated to be solved [12].

The general strategy to model a real problem follows the steps which are shown in figure 2.1.

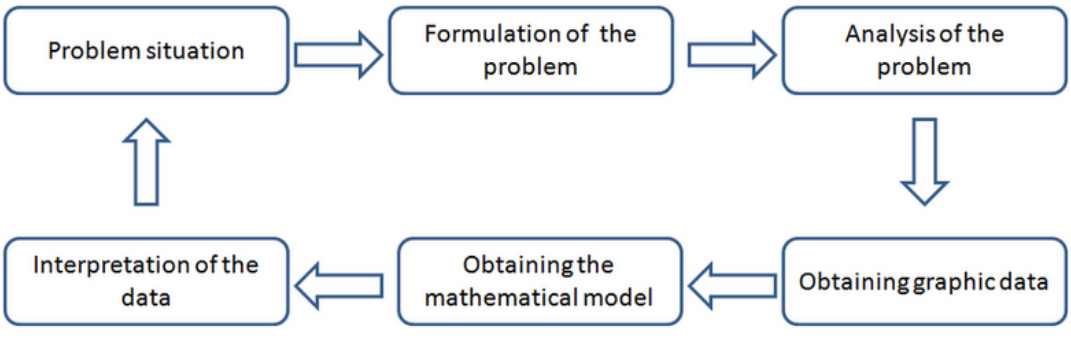


Figure 2.1: General steps in modelling [13].

Modelling strategy is made up of a closed cycle where the problem situation is formulated, analyzed, simulated and compared with the real problem Then, it is represented through mathematical equations and simulated with computing tools.

2.1 Modeling of Biosystems

Ordinary differential equations are used to describe bioprocesses. These equations are used to represent the change over time of the concentration of external metabolites.

To understand this concept, a simple reaction is taken.

$$A + B \rightarrow X. \tag{2.1}$$

The concentrations of metabolites A and B are decreasing as fast as the concentration of the metabolite X is increasing. The reaction rate, which describes how fast the concentration of a metabolite is increasing or decreasing, is defined as,

$$r_i = -\frac{dA}{dt} = -\frac{dB}{dt} = \frac{dX}{dt}. \tag{2.2}$$

This reaction rate is used to describe the change of concentration of an external metabolite i over time which is described by

$$\frac{d(c_i V_{culture})}{dt} = r_i V_{culture} - (c_{i_{in}} \dot{V}_{in} - c_{i_{out}} \dot{V}_{out}). \tag{2.3}$$

The equations that are used to model a bioprocess depends on the type of bioreactor. The bioprocess takes place in a continuous, batch or fed-batch-bioreactor. Almost in all biosytems there is an exchange of mass between the liquid and gas phase and vice versa. For instance, when a biosystem is running in an aerobic way is necessary to transfer the oxygen from the gas to the liquid and the carbon dioxygen from the liquid to the gas. Therefore, to represent properly a biosystem, the model has to include the physical principles of mass transfer.

2.1.1 Batch process

In the batch process, the culture medium, which contents the carbon source and other minerals and vitamins, is mixed inside the reactor with micro-organism. During a determined period of time the concentration o biomass as wells as the concentrations of the carbon source and the rest of nutrients vary. At the end of this process the both of the reactor is discharged.

This kind of tanks are equipped with an agitator, a heating or cooling system and with a buffer solution which enables to control the pH value in the broth. The mode of operation

of a batch reactor are shown in figure 2.2.

Figure 2.2: Stirred tank reactor[14].

They are most common types of aerobic bioreactors due to they can be used with a large variety of micro-organism. Within this reactor the concentration of the different metabolites as well as the temperature, the pressure, the pH value and the aeration rate can be controlled.

To model a batch reactor is assumed to be well stirred with a uniform concentration distribution across the reactor. It can be modelled by

$$\frac{d(VX)}{dt} = r_x V \tag{2.4}$$

$$\frac{d(VS)}{dt} = -r_s V \tag{2.5}$$

$$\frac{d(VP)}{dt} = r_p V. \tag{2.6}$$

Where $\dot{V}$ is the volume flow and X, s and p are the concentrations of biomass, substrate and product.

2.1.2 Continuous stirred reactor

In a stirred reactor, the medium is added continuously to the reactor. Inside the reactor a mechanical agitation is used to maintain a constant and homogeneous concentration. Due to it is a continuous process, the culture inside the reactor is removed with the same flow as the feed to maintain the steady-stead. Continuous process is used for high culture volume. As the batch reactor, stirred reactors are equipped with a submerged aerator, a thermal

jacket and with different sensors to control metabolites concentrations inside the reactor. The mode of operation of a continuous stirred reactor is shown in figure 2.3.

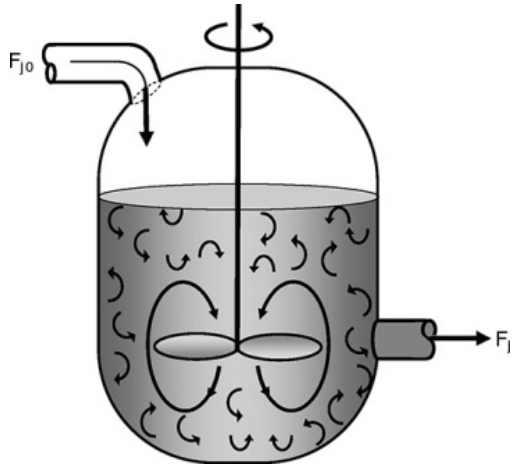


Figure 2.3: Continuous bioreactor[14].

In this case, the process can be modelled without differential equations due to the fact that the concentration of the metabolites within the reactor is constant over time such that equations can be simplified

$$\dot{V}X = r_x V \tag{2.7}$$

$$\dot{V}(s - s_{in}) = -r_s V \tag{2.8}$$

$$\dot{V}p = r_p V. \tag{2.9}$$

Where s_{in} and are the concentrations of substrate of the volume flow that is fed to the reactor.

2.1.3 Fed-batch reactor

The process is an intermediary between a batch and a continuous reactor. In this process additional nutrients are added progressively to the reactor during cultivation. The products remain in the bioreactor until the end of the run when the reactor is discharged. This reactor is commonly used because it enables to obtain better yields and higher biomass concentration at the end of the process. The features of this kind of reactor are shown in figure 1.9.

The equations which describe a fed-batch reactor are

$$\frac{d(VX)}{dt} = r_x V + \dot{V} X_0 \tag{2.10}$$

$$\frac{d(VS)}{dt} = -r_s V + \dot{V} s_0 \tag{2.11}$$

$$\frac{d(VP)}{dt} = r_p V + \dot{V}. \tag{2.12}$$

Where X_0, s_0, p_0 are the initial concentration of biomass, substrate and product.

2.1.4 Mass transfer

Mass transfer describes the transport of mass from one point to another one due to a driving force. Mass transfer can be

1. Molecular. It is based on an individual movements of molecules.
2. Convective. It is based in the global movement of a fluid.

In the molecular mass tranfer, mass can be transferred between same and different phases. Transfer of material between different phases is important in most biosystems. When mass is transferred from one phase to another across an interface that separates both, the resistance to mass transfer in the interface causes a gradient of concentration. The concentration profile for a gas-liquid near the interface is shown in figure 2.4.

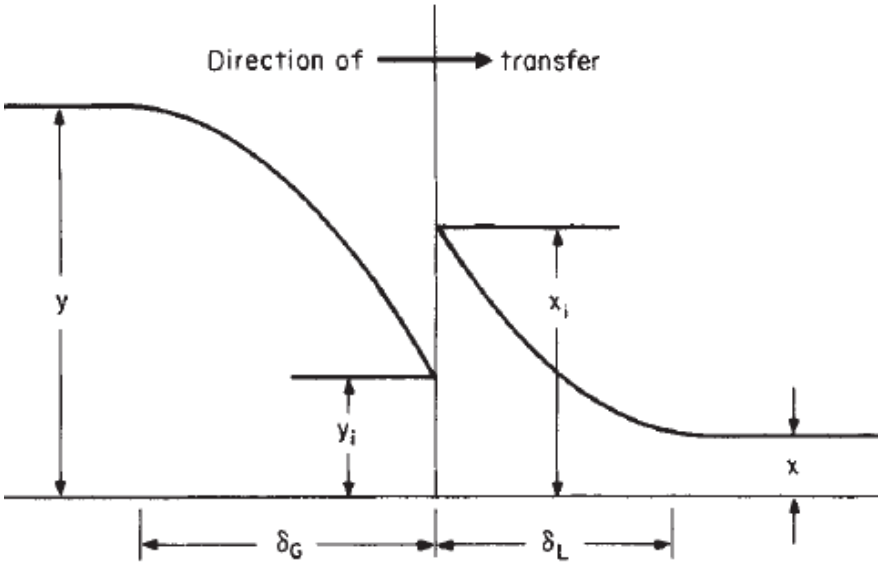


Figure 2.4: Concentration gradients near a interface[15].

The concentrations of the diffusing compound in the both sides of the interface are unequal. These concentration can be related to each other by using the laws of thermodynamic equilibrium. For instance, the concentration in a gas-liquid mass transfer can be figured out from Henry law that is expressed by

$$c = pk_H. \tag{2.13}$$

Where p is the partial pressure of the gas in the interface, c is the concentration of the same component in the liquid and k_H is the Henry constant.

It is assumed that the thermodynamic equilibrium is reached at the gas-liquid interface almost immediately when a gas and a liquid are brought into contact.

Gas-liquid mass transfer

For systems where there is a mass transfer between a liquid and a gas, the rate at which mass moves from one point to another one can be expressed by equations where the rate of mass transfer is proportional to the difference between the bulk concentration and the concentration at the interface [15]

$$N_A = k_G(p - p^*) = k_L(c^* - c). \tag{2.14}$$

Where N_A is the mass-transfer rate, k_G is the mass-transfer coefficient in the gas, k_L is the mass-transfer coefficient in the liquid, p is the solute partial pressure in bulk gas, p^* is the solute partial pressure at the interface, c is the solute concentration in the bulk liquid, and c^* is the solute concentration in liquid at interface.

Gas-liquid mass transfer is an important factor in the model of this thesis because it is an aerobic process. Cells in an aerobic culture take up the oxygen which is dissolved in the broth. The rate of oxygen transfer from gas to liquid is therefore of prime important to ensure metabolism of cells and therefore growth of cells.

The solubility of oxygen in aqueous solutions at ambient temperature and pressure is only about 10 ppm [16].

The rate at which oxygen is consumed by the cells in a fed-batch reactor determines the rate at which it has to be transferred from gas to liquid. Many factors influence oxygen demand such as cell species, culture growth phase, and nature of the carbon source in the medium.

Phase distribution and mass transfer mechanism

To model the transfer of oxygen between from the gas phase to the cells and vice versa it is necessary to know the different mechanism that govern the mass transfer.

In a fed-batch reactor, oxygen is sparged from the bottom of the reactor, creating in the liquid a mass of gas bubbles that exchange mass along the reactor. Figure 2.5 is shown as a example of a sparged fed-batch reactor.

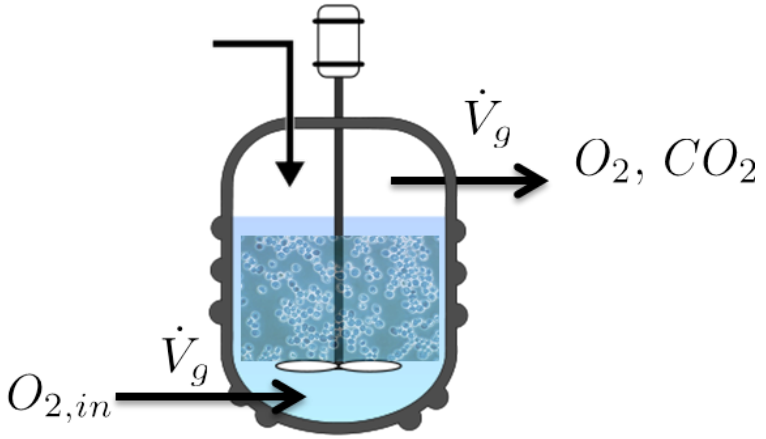


Figure 2.5: Phase distribution in the fed-batch reactor.

Therefore, gas molecules must overcome a series of transport resistances in the mass transfer process. To understand the whole process, Tthe transfer of oxygen is taken from the gas phase to cells as is shown in figure 2.6.

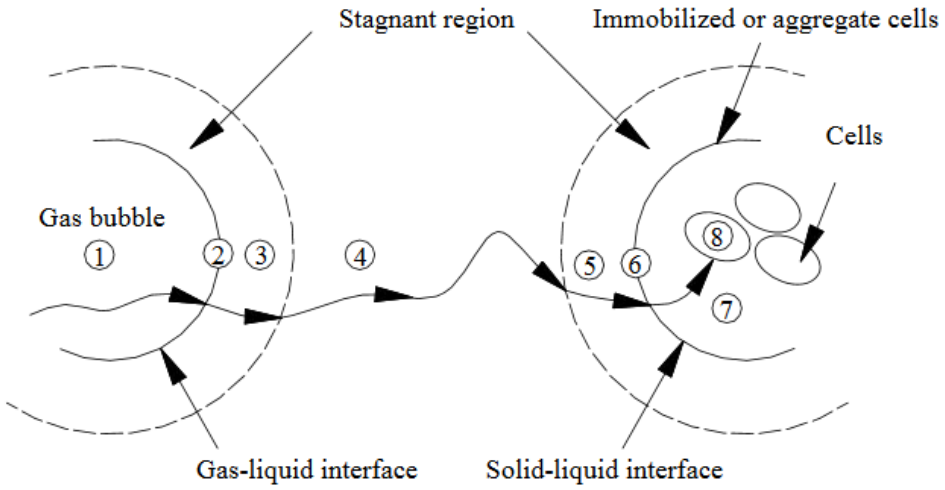


Figure 2.6: Steps for oxygen transport from gas bubble to cell [16].

As it can be seen in the previous figure, oxygen molecules must overcome 8 mass transport resistances to be transfer from the bulk of the gas phase to the site where oxygen is used in intracellular reactions [16].

1. Transfer of oxygen from the bulk gas to the gas-liquid interface which is relatively fast.

2. Transport across the gas-liquid interface.

3. Diffusion of oxygen from the gas-liquid interface to the well-mixed bulk liquid which is the major resistance to oxygen transfer.

4. Transport of oxygen trough the liquid to a relative unmixed liquid region surrounding the cells. In a well mixed fermenter, concentration gradients in the liquid are minimized and mass-transfer resistance in this region are small.

5. Diffusion through the stagnant region of cells.If the cells form chumps, this resistance can be significant.

6. Transport through the cell-liquid interface. This resistance is generally neglected.

7. Diffusion until the wall os the cells. This resistance depends on the distribution of the cells. If cells are in the broth distributed in clumps, intraparticle resistance can be significant due to oxygen has to diffuse through cells clumps to reach the interior of cells. The significance of this mass transfer resistance depends on the size of the clumps.

8. Transport inside cells until the intracellular reaction site. Intracellular oxygen-transfer resistance is negligible because of the small distances involved.

Overall mass transfer coefficient

All the transport resistances should be considered in the model. It would be too complicated to model all the steps of the mass transfer due to it would be necessary to know the concentration in the interfaces of all the mass transfer resistances. To solve this problem it is necessary to take an overall mass transfer coefficient which represents all resistances. The coefficient can be calculated by

$$\frac{1}{K} = \frac{1}{k_1} + \frac{1}{k_2} + \frac{1}{k_3} + \frac{1}{k_4} + \frac{1}{k_5} + \frac{1}{k_6} + \frac{1}{k_7} + \frac{1}{k_8}. \tag{2.15}$$

And the expression for the mass transfer is

$$N_A = K_G(p - p^*) = K_L(c^* - c). \tag{2.16}$$

In this case, c^* is the equilibrium concentration in the liquid phase and c is the concentration in the liquid phase.

In this thesis, certain errors are assumed using an overall mass transfer coefficient due to that some mass transfer resistances are neglected. Nevertheless, these errors enable to formulate a more simple model.

2.2 General balance

Once the features of a fed-batch reactor, the phase distribution in the reactor and the physical principles of the mass gas-liquid mass transfer are know it is possible to formulate the general balances.

The general balances will are divided in three parts. The first part includes the general material balance for the concentration of substrates inside the fed-batch reactor. The second, includes the balance for a gas component in the liquid. The third includes the balance for a component in the gas phase[17].

1. General balance for external metabolites.

$$\frac{d(c_i V)}{dt} = r_i V - c_{i_{in}} \dot{V} \tag{2.17}$$

$$\frac{dV_R}{dt} = \dot{V}. \tag{2.18}$$

   Where c_i is the concentration of ethanol, biomass or glucose inside the reactor, V is the volume of the culture in the reactor, $\dot{V}$ is the volume flow that is fed to the reactor, r_i is the reaction rate and $c_{i_{in}}$ is the concentration in of the metabolites in $\dot{V}$.

2. Balance for gas components in the liquid.

$$\frac{dc_{i,l}}{dt} = r_i X + k_{l,i}({c_{i,l}}^* - c_{i,l}). \tag{2.19}$$

   Where $c_{i,l}$ is the concentration of oxygen or carbon dioxygen in the liquid that is being produced or consumed by cells, X.

3. Balance for gas components.

$$\frac{dc_{i,g}}{dt} = -k_{g,i}({c_{i,l}}^* - c_{i,l}) - \frac{\dot{V}_g}{V_R} c_{i,g}. \tag{2.20}$$

Where V_g is the volume flow of gas that is fed to the reactor and $c_{i,g}$ can be the concentration of oxygen or carbon dioxygen in the gas phase.

The reaction rates can be described by a composite Monod kinetic which is given by equation 2.21.

$$r_i = r_{max} \prod_i \frac{c_i}{K_i + c_i}. \tag{2.21}$$

Where r_{max} is the maximal velocity of the reaction, K_i are the Monod constants and c_i are the concentrations of the substrates of the reaction.

The inhibition of a reaction is described by

$$r_{inh} = \frac{K_I}{K_I + c_i}. \tag{2.22}$$

Where K_I is the inhibition constant.

2.3 Elementary modes and stoichiometric network

In biochemistry a stoichiometric network is a series of reactions where an initial substrate is converted into a final product through a series of intermediate metabolites. The formulation of a model which contents the thousand of intermediate metabolites and enzymes is a difficult task and sometimes the lack of information of the resulting mathematical models involves a bad model development.

A tool that has the potential to solve this lack of information is Elementary Modes (EMs). EMs is a analysis tool to characterize cellular metabolism that enables to identify the smallest sub unit in a network that can support cellular functions in steady state [18]. A simple example is shown in figure 2.7.

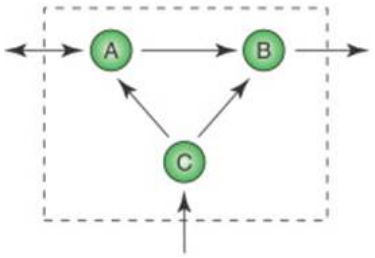


Figure 2.7: Symple network.

There are four possible pathways in this network that can run in steady state which is described in figure 2.8.

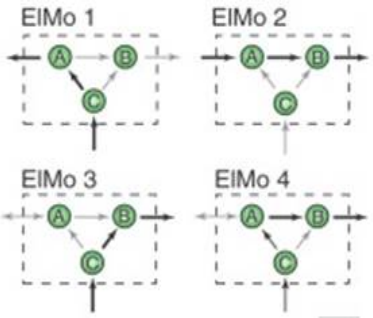


Figure 2.8: Elementary modes of a symple network.

In this thesis, the glycolysis, the TCA cycle and the pentose phosphate pathway are considered to build the network of the *Saccharomyces cerevisiae*. This network is shown in Figure 2.9.

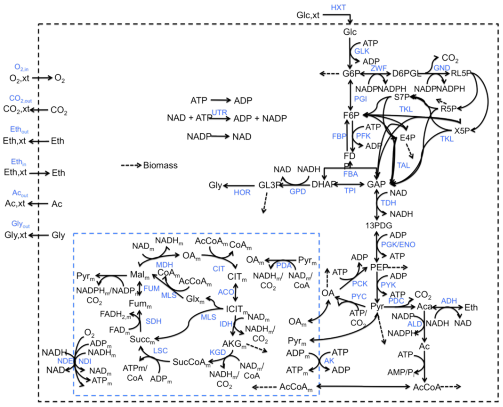


Figure 2.9: *Saccharomyces cerevisiae* network.

Chapter 3

Results

In this chapter the theoretical principles explained in chapter 2 are implemented. In the first section, the elementary modes of *Saccharomyces cerevisiae* during the growth are presented.

In the second section, the dynamic model is explained. The dynamic model is expected to describe the ethanol production caused by anaerobic respiration.

The next section describes the validation of the model parameters needed to represent properly the anaerobic respiration and the results of the simulation.

Finally, it is necessary to optimize the obtained results to reduce the ethanol production. The experimental data which is used in this thesis is derived by the VH Berlin.

3.1 Elementary modes of *Saccharomyces cerevisiae*

Elementary models are used to describe the growth of *Saccharomyces cerevisiae* over time. Before starting with the description of elementary modes it is necessary to define the products and the substrates of the network. Glucose, O_2 and ethanol are used as substrates in the metabolism of *Saccharomyces cerevisiae* where the products are ethanol and CO_2. The energy metabolites, ATP and ADP, are responsible of activating or inhibiting the reactions of the network.

For the reaction of oxygen, it is assumed that the reaction takes place in the cells so that

the need of NADH is neglected. The result is

$$0.5\ ATP + 0.5\ O_2 = 1.5\ ADP. \tag{3.1}$$

The growth of the cells is characterized by producing biomass which can be summarized by

$$1\ Glc + w_{ATP}\ ATP = 0.39\ CO_2 + 1.4\ BM + w_{ATP}\ ADP. \tag{3.2}$$

Where w_{ATP} is chosen as a weighting factor.

As it was described in chapter 2, ethanol can be produced via glycolysis or via pentose phosphate pathway. Both reactions are

$$1\ Glc + 2\ ADP = 2\ ATP + 2\ Eth + 2\ CO_2 \tag{3.3}$$

$$1\ Glc + 1.67\ ADP + 2\ NADP = 2.67\ CO_2 + 1.67\ Eth + 1.67\ ATP + 2\ NADPH \tag{3.4}$$

Where reaction 3.3 is responsible for ethanol production via glycolysis while reaction 3.4 is responsible via the pentose phosphate pathway. For this thesis the production of ethanol via pentose phosphate pathway is neglected and only reaction 3.3 is considered.

When ethanol is used as a substrate to produce biomass it must be converted back to glucose-6-phosphate which leads to a reduced yield of biomass. The overall reaction for the consumption of ethanol can be described by

$$1\ Eth + w_{ATP_1}\ ATP = 0.35\ CO_2 + 0.41\ BM + w_{ATP_1}\ ADP. \tag{3.5}$$

Where w_{ATP_1} is chosen as a weighting factor.

To guarantee an exchange of ATP and ADP, the reaction of the adenylate kinase is added to the system

$$1\ ATP = 1ADP. \tag{3.6}$$

3.2 Oxygen distribution

It is known that ethanol can be produced due to the Crabtree effect or due to anaerobic respiration. When ethanol is produced due to anaerobic respiration, the oxygen concentration

inside the bioreactor is too low to supply all cells so it is necessary to know how the oxygen is distributed.

In this thesis is assumed that the oxygen is distributed in different phases.

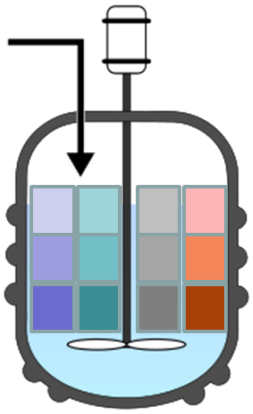
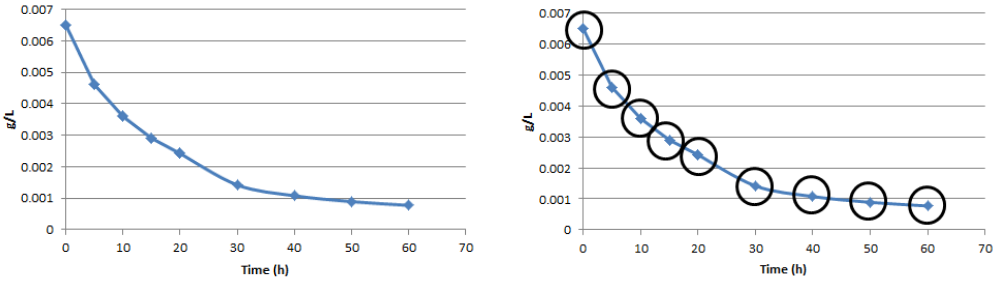


Figure 3.1: Oxygen evolution.

The oxygen is fed from the bottom of the bioreactor and it is consumed along the volume of the culture. The concentration of oxygen decreases along the bioreactor which create a phase distribution as is shown in figure 3.1. The more intensive colours indicate the areas of the bioreactor where the oxygen concentration is higher.

To implement an oxygen distribution in the model, it is assumed that there is a certain number of phases. Therefore at any point of time a different concentration of oxygen is assigned to each phase. figure 3.2 shows an example where the oxygen distribution is implemented.

The oxygen concentration is calculated by a random value which is added to the oxygen



(a) Homogeneous phase distribution (b) Non Homogeneous phase distribution

Figure 3.2: Phase distribution

current oxygen concentration which is described by

$$O_2' = O_2 + N(0, 0.0005). \tag{3.7}$$

N stands for a noprmal distribution with mean 0 and standard deviation 0.0005.

3.3 Dynamic model

The dynamic model follows the principles that are explained in chapter 2, the elementary modes that are described in section 3.1 and the oxygen distribution that is presented in section 3.2. With respect to the oxygen distribution, it is necessary to calculate for each phase, the energy metabolites concentration and the reaction rates. A vector is created whose number of components is equal to the number of phases which are assumed inside the bioreactor. To calculate the balance of the external metabolites, is considered the sum of all phases.

The model will be divided in

1. External metabolites.

   These metabolites are glucose, ethanol and biomass. The change of concentration over time of glucose is

   $$\frac{dGlc}{dt} = -\left(\frac{1}{N}\sum_{i=1}^{N}\mu(i)\right)X - \left(\frac{1}{N}\sum_{i=1}^{N}r_{Eth}(i)\right)X + D(Glc_{in} - Glc). \tag{3.8}$$

   Where D is the dilution rate, $\underline{\mu}$ and $\underline{r}_{Eth}$ are reaction rates, N is the number of phases which are assumed to divide the bioreactor.

   The change of ethanol is

   $$\frac{dEth}{dt} = -\left(\frac{1}{N}\sum_{i=1}^{N}\mu_{Eth}(i)\right)X + 2\left(\frac{1}{N}\sum_{i=1}^{N}r_{Eth}(i)\right)X - DEth. \tag{3.9}$$

   The growth of biomass is

   $$\frac{dX}{dt} = a\left(\frac{1}{N}\sum_{i=1}^{N}\mu_{Eth}(i)\right)X + b\left(\frac{1}{N}\sum_{i=1}^{N}\mu(i)\right)X - DX. \tag{3.10}$$

Where a and b represent the yield of cells using ethanol or glucose as substrate. $\underline{\mu}_{Eth}$ is the reaction rate for ethanol consumption.

The change of the concentration of O_2 is

$$\frac{dO_{2,g_{out}}}{dt} = -k_{l,O_2}(O_2^* - O_{2,l}) + \frac{\dot{V}_g}{35-V}O_{2,g_{in}} - \frac{\dot{V}_g}{35-V}O_{2,g_{out}}. \tag{3.11}$$

Where $O_{2,g_{in}}$ and $O_{2,g_{out}}$ are the concentration of oxygen of the gas flow at the entrance and exit of the reactor.

The concentration of CO_2 is

$$\frac{dCO_{2,g_{out}}}{dt} = -k_{l,CO_2}(CO_2^* - CO_{2,l}) + \frac{\dot{V}_g}{35-V}CO_{2,g_{in}} - \frac{\dot{V}_g}{35-V}O_{2,g_{out}}. \tag{3.12}$$

2. Gas components in the liquid.

   The change of oxygen in the liquid is

$$\frac{dO_{2,l}}{dt} = -\left(\frac{1}{N}\sum_{i=1}^{N} r_{O_2}(i)\right) X + k_{l,O_2}(O_2^* - O_{2,l}). \tag{3.13}$$

   Where $\underline{r}_{O_2}$ is the reaction rate for the oxygen uptake. The production of CO_2 is

$$\begin{aligned}\frac{dCO_{2,l}}{dt} = 0.35&\left(\frac{1}{N}\sum_{i=1}^{N}\mu_{Eth}(i)\right)X + 0.39\left(\frac{1}{N}\sum_{i=1}^{N}\mu(i)\right)X \\ &+ 2\left(\frac{1}{N}\sum_{i=1}^{N} r_{Eth}(i)\right)X + k_{l,CO_2}(CO_2^* - CO_{2,l}).\end{aligned} \tag{3.14}$$

3. Energy metabolites

   ADP and ATP are described by

$$\frac{d\underline{ATP}}{dt} = 2\underline{r}_{Eth} + \underline{r}_{AK} - w_{ATP}\underline{\mu} + 1.5c\underline{r}_{O_2} - w_{ATP_1}\underline{\mu}_{Eth} \tag{3.15}$$

$$\frac{d\underline{ADP}}{dt} = -2\underline{r}_{Eth} - \underline{r}_{AK} + w_{ATP}\underline{\mu} - 1.5c\underline{r}_{O_2} + w_{ATP_1}\underline{\mu}_{Eth}. \tag{3.16}$$

   Where $\underline{r}_{AK}$ is the reaction rate for $\underline{ATP}$ and $\underline{ADP}$ and w_{ATP} and w_{ATP_1} are the weightings factors.

4. reaction rates

These reaction rates result from the reduced stoichiometric network presented in section 3.1. The reaction rate for the oxygen consumption is described by

$$\underline{r}_{O_2} = r_{O_{2,max}} \left(\frac{(O_2 + \underline{R})}{K_{O_2} + (O_2 + \underline{R})} \right) \left(\frac{\underline{ADP}}{K_{ATP_{O_2}} + \underline{ADP}} \right). \tag{3.17}$$

Where $r_{O_{2,max}}$ is the maximum rate at which O_2 can be consumed and $\underline{R}$ is the vector of the random values

The growth rate is described by

$$\underline{\mu} = \mu_{max} \left(\frac{Glc}{K_{Glc_\mu} + Glc} \right) \left(\frac{\underline{ATP}}{K_{ATP_\mu} + \underline{ATP}} \right). \tag{3.18}$$

Where μ_{max} is the maximum growth rate of the cells, which is assumed to be equal for all cells. The reaction rate for producing ethanol is

$$\underline{r}_{Eth} = r_{Eth,max} \left(\frac{\frac{Glc}{K_{Glc_{Eth}} + Glc}}{1 + \left(\frac{K_{ATP_{Eth}}}{\underline{ADP}} \right)^n} \right). \tag{3.19}$$

Where $r_{Eth,max}$ is the maximum rate of ethanol production and n a parameter. The reaction for ethanol consumption can be described by

$$\begin{aligned} \underline{\mu}_{Eth} = \mu_{Eth,max} &\left(\frac{Eth}{K_{Eth_\mu} + Eth} \right) \left(\frac{\underline{ATP}}{K_{ATP_{\mu,Eth}} + \underline{ATP}} \right) \\ &\left(\frac{\frac{K_I}{K_I + Glc}}{1 + \exp^{-1000(\underline{r}_{O_2} - 0.003)}} \right). \end{aligned} \tag{3.20}$$

Where $\mu_{Eth,max}$ is the maximum consumption rate of ethanol. The reaction of ATP to ADP is described by

$$\underline{r}_{AK} = r_{AK,max} \underline{ADP}^2 \left(1 - \frac{\underline{ATP}}{K_{ATP} \underline{ADP}^2} \right). \tag{3.21}$$

Where $r_{AK,max}$ is the maximum rate at which ATP is converted to ADP.

3.3.1 Mass transfer coefficient

As the concentration in each interface, shown in Figure 2.6, from gas phase to cells is unknown, it is necessary to use a global mass tranffer coefficient. The global mass transfer coefficient neglects some transport resistances and assumes that all mass transfer resistance takes place between the liquid and the gas phase of the bubbles. Therefore, some errors are assumed in the formulation of the model.

To calculate the value of the global mass transfer coefficient it is required to find a correlation which describe the behaviour of a sparged fed-batch bioreactor. This correlation is described by [15]

$$k_{l,i} = \alpha \left(\frac{P}{V} \right)^{\beta} \left(\frac{\dot{V}_g}{A} \right)^{\gamma}. \tag{3.22}$$

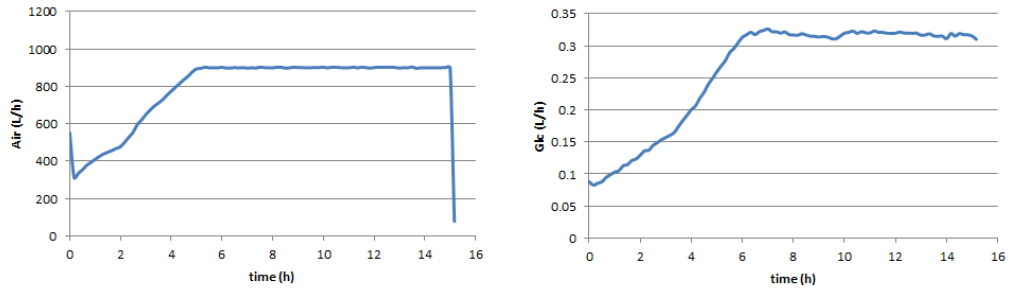
This correlation is used to modelate the mass tranfer of O_2 and CO_2.

In this correlation appears two new variables. P is the stirring power of the biosystem in watts and A is the transversal area of the bioreactor in dm. Both variables are known from the experimental data of the biosystem. $\dot{V}_g$ is the inlet gas flow in L/h and V is the volume of the culture in l.

3.3.2 Parameter estimation

The parameter estimation is done in gPROMS with the experimental data provided by VH Berlin. Before showing the result of the parameter estimation it is necessary to describe the experiment.

The experiment was carried out in a fed-batch reactor. The controllable variables were the aeration rate and the glucose feed rate which are shown in figure 3.3.



(a) Aeration rate in the experimental data (b) Glucose in the experimental data

Figure 3.3: Phase distribution

The aeration rate during the experiment was increased due to that the cells increase. The glucose feed rate increases as well.

The measured variable in the experiment was the concentration of ethanol which is shown in figure 3.4.

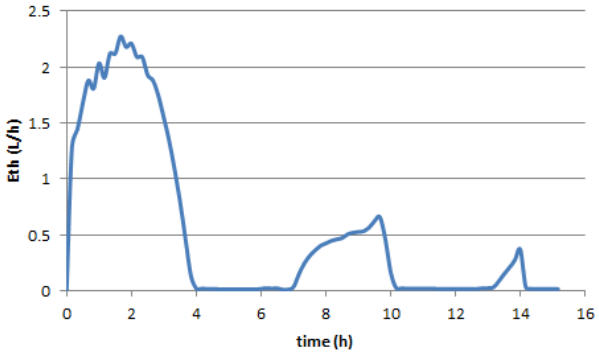


Figure 3.4: Glucose in the experimental data.

In the experiment, ethanol was produced by the Crabtree effect and in anaerobic respiration.

The initial values, bounds and final values of the parameters are summarized in table 3.1.

Parameter	Value	Lower bound	Upper bound
a	4.98375	1E-8	10
α_{CO_2}	0.68	1E-8	1
ADP_0	13,763	0.1	20
a_{O_2}	0.556428	1E-8	1
ATP_0	15.2915	0.1	20
b	0.276213	1E-8	5
c	60723.9	1E-8	1E8
k	3.42754	1E-8	8
K_{ATP}	2.8833	1E-8	5
k	3.42754	1E-8	8
$K_{ATP_{Eth}}$	0.252671	1E-8	3
K_{ATP_μ}	0.000623384	1E-8	5
$K_{ATP_{\mu,Eth}}$	0.134277	1E-8	1
$K_{ATP_{O_2}}$	0.243211	1E-8	1
K_{Eth_μ}	4.10421E-6	1E-8	1
$K_{Glc_{Eth}}$	0.00736133	1E-8	1
K_{Glc_μ}	1.97098	1E-8	2
K_I	2.81409	1E-8	6
K_{O_2}	0.668212	1E-8	1
β_{CO_2}	0.02	1E-8	1
β_{O_2}	0.438629	1E-8	3
γ_{CO_2}	0.8	1E-8	1
γ_{O_2}	0.00787992	1E-8	2
$\mu_{Eth,max}$	0.115767	1E-8	1
μ_{max}	2.12367	1E-8	5
n	3.73714	3	10
$r_{AK,max}$	1.38319	1E-8	3
$r_{Eth,max}$	0.892875	1E-8	3
$r_{O_2,max}$	0.0602912	1E-8	0.5
w	0.33	1E-8	2
w_1	0.2	1E-8	2
w_{ATP}	19.3198	1E-8	50
w_{ATP_1}	4.48872	1E-8	10

Table 3.1: Parameter estimation.

After the parameter estimation the comparison between the experimental and the predicted values is shown in figure 3.5.

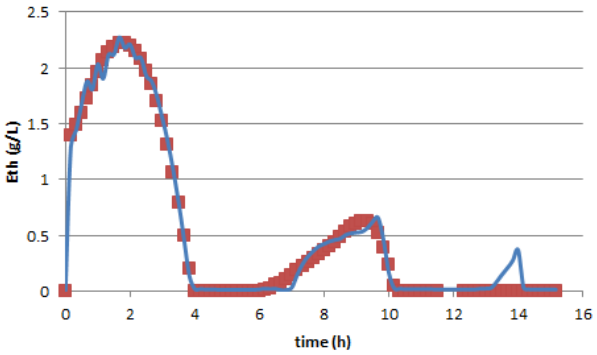


Figure 3.5: Measurements plot.

As it can be seen in figure 3.5 the parameters describe the production of ethanol from 0 to 4 hour and from 7 to 10 hour produced by the Crabtree effect. The parameters are not be able to represent the production of ethanol from 13 to 14 hour which is caused by anaerobic respiration.

3.4 Model simulation

In this section the results of the model simulation are shown. Three different results are presented. In each, only the inputs, glucose, biomass and ethanol concentrations and the reaction rates are represented. The rest of concentrations are shown in Appendix A.

3.4.1 Homogeneous distribution of oxygen

In this simulation, it is assumed an homogeneous concentration in the bioreactor as it is shown in figure 3.2(a). The number of phase of the model is in this case $N = 1$. The parameters of this model are taken from a previous master work.

The initial condition are summarized in table 3.2.

Parameter	Value(g/L)
	4.98375
ADP_0	4.69
ATP_0	0,1
Glc_0	0.2
X_0	12.5
O_{2_0}	0.0065 5
CO_{2_0}	1.2

Table 3.2: Initial conditions.

The aeration rate is set constant along the simulation with 900 L/h. The glucose rate is increased over time which is shown in figure 3.6

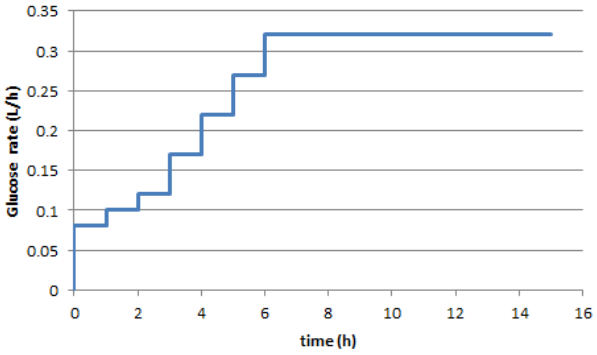
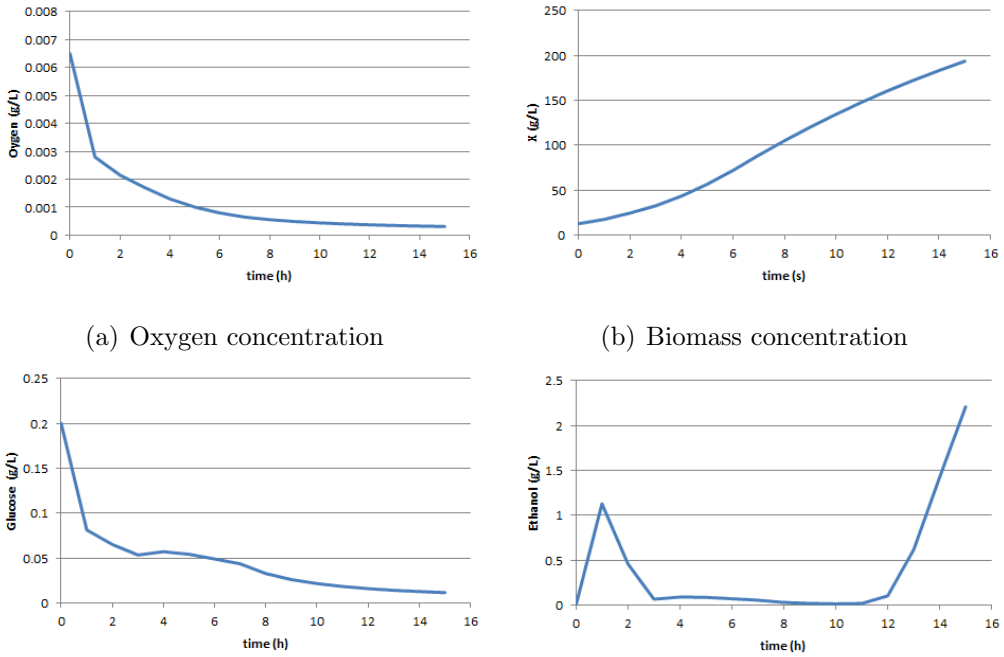


Figure 3.6: Glucose rate.

The evolution of the external metabolites are shown in figure 3.7



(a) Oxygen concentration

(b) Biomass concentration

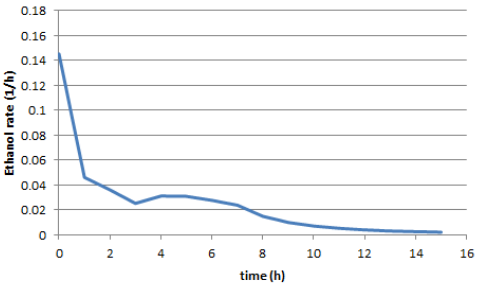
(c) Glucose concentration

(d) Ethanol concentration

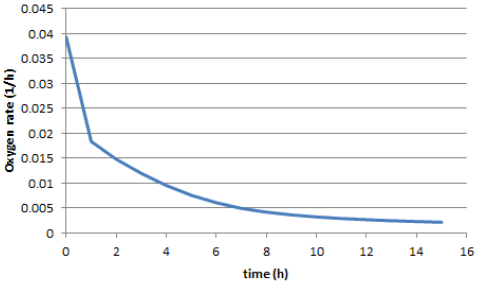
Figure 3.7: Concentration of the external metabolites.

In the 12 hour of the simulation there is a change from a aerobic respiration to anaerobic respiration. The result is the synthesis of ethanol. Ethanol is not consumed so the model is not able to represent the production of ethanol due to air limitation.

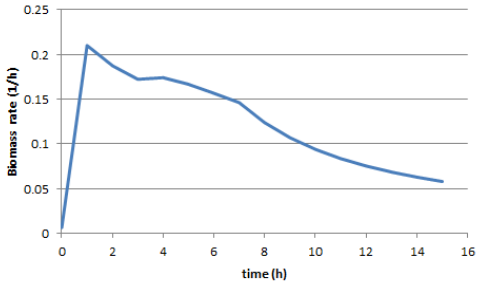
r_{Eth}, μ and r_{O_2} are shown in figure 3.8.



(a) Ethanol reaction rate



(b) Oxygen reaction rate

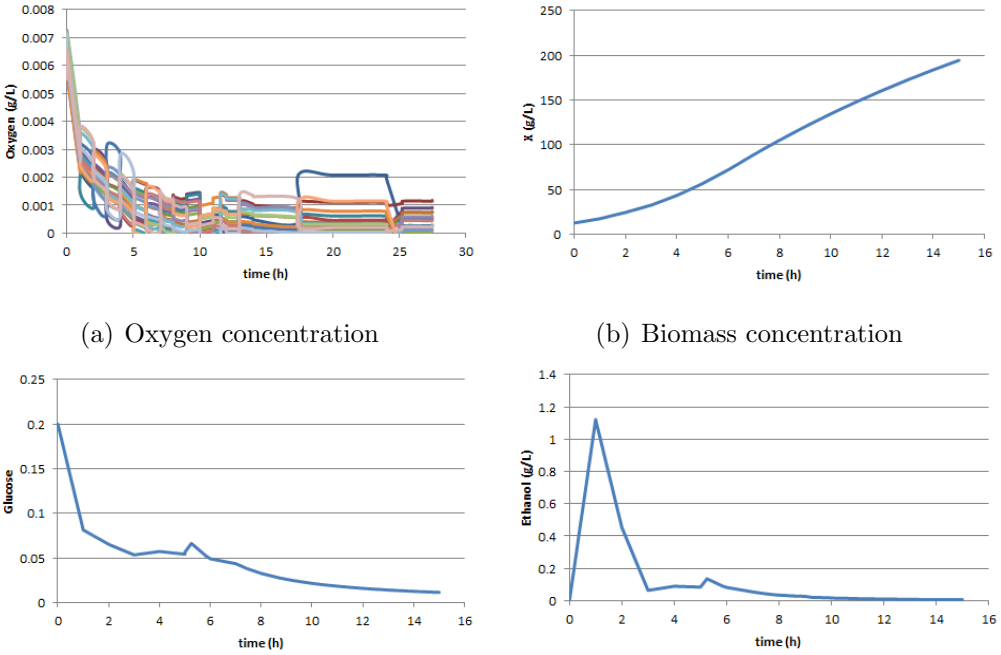


(c) Biomass reaction rate

Figure 3.8: Reaction rates.

3.4.2 Distributed concentration of oxygen

The oxygen distribution is simulated with $N = 20$. The intial conditions are the same shown in Table 3.2 and the aeration rate is 900 L/h. The results of the external metabolites are shown in figure 3.9.



(a) Oxygen concentration

(b) Biomass concentration

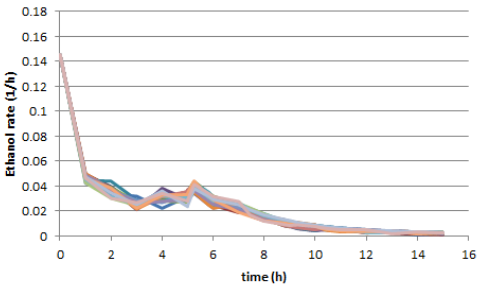
(c) Glucose concentration

(d) Ethanol concentration

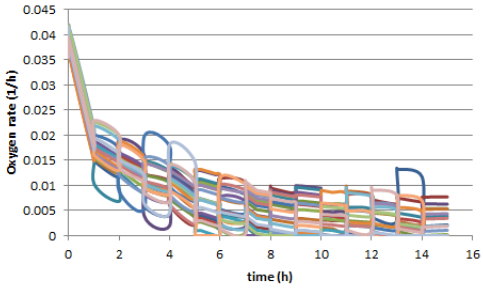
Figure 3.9: Concentration of the external metabolites.

When the oxygen in the liquid is too low, after 9 hours, the cells do not produce ethanol comparing to the other simulation. Therefore, this model is not able to simulate the production and consumption due to air respiration.

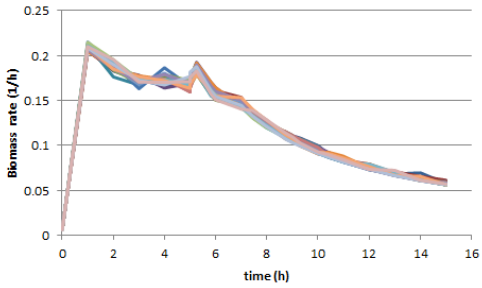
r_{Eth}, μ and r_{O_2} are shown in figure 3.10.



(a) Ethanol reaction rate



(b) Oxygen reaction rate



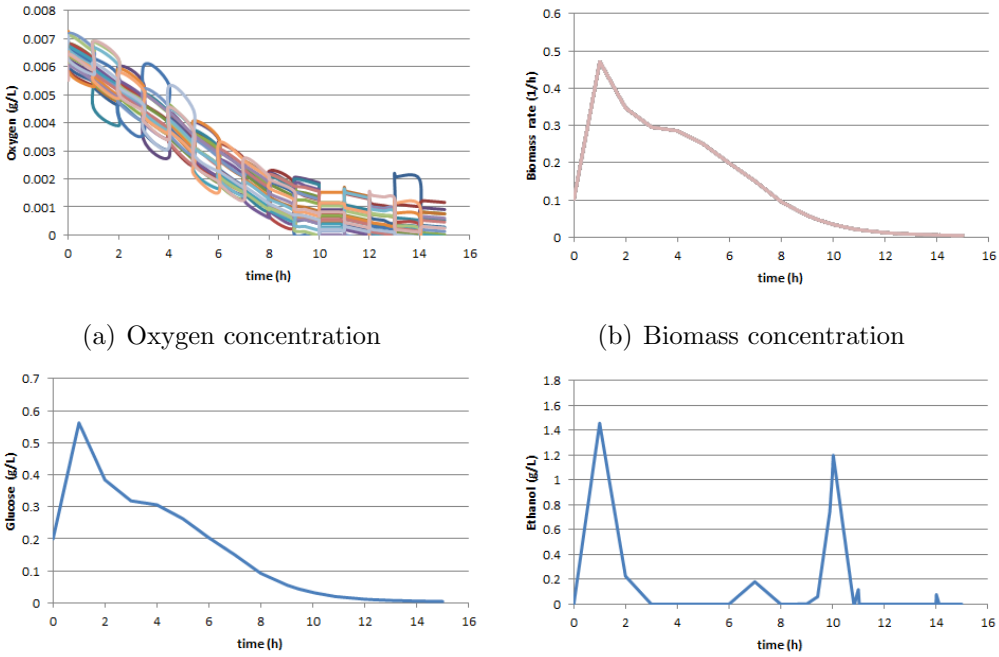
(c) Biomass reaction rate

Figure 3.10: Reaction rates.

3.4.3 Parameter estimation implemented

The parameter estimated in section 3.3.2 are implemented in the model for the next simulation.

The initial conditions are summarized in table 3.2, the aeration rate is 900 L/h and $N = 20$. The glucose rate is shown in figure 3.11.



(a) Oxygen concentration

(b) Biomass concentration

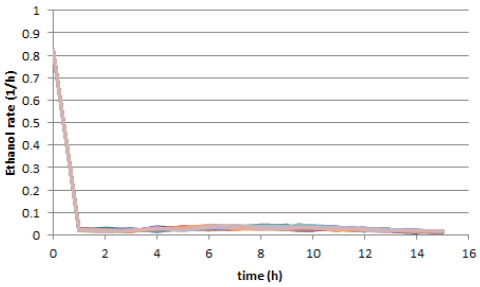
(c) Glucose concentration

(d) Ethanol concentration

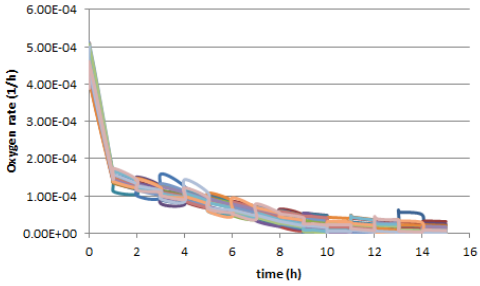
Figure 3.11: Concentration of the external metabolites.

The model simulates the ethanol production due to the Crabtree effect from 0 to 3 hours and from 6 to 8 hours as well as the ethanol production due to air limitation from 9 to 11 hours. The model is able to simulate the experimental data.

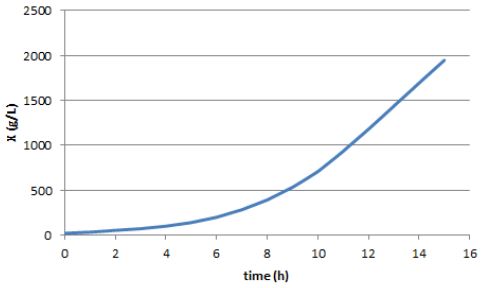
r_{Eth}, μ and r_{O_2} are represented in Figure 3.12.



(a) Ethanol reaction rate



(b) Oxygen reaction rate



(c) Biomass reaction rate

Figure 3.12: Reaction rates.

3.5 Optimization

Mathematical optimization is the selection of the best option from a set of available alternatives. Considering the model of a process, an optimization problem consists in maximizing or minimizing an objective function by choosing an input, called control variable, from a set of possibilities and computing the value of the function as is described by

$$\min/\max\ z = f(x). \tag{3.23}$$

The optimization problem can be subject to constrains or bounds

$$\text{Constraints} \quad g_i(x) \leq 0 \forall \rightarrow i = 1, ..., M \tag{3.24}$$

$$\text{Bounds} \quad lb_k \leq x_k \leq ub_k \forall \rightarrow k = 1, ..., n. \tag{3.25}$$

Control variable can be divided in four types:

1. Piecewise-constant controls. Control variable remains constant until a certain instant

of time when it jump discretely to another value.

2. Piecewise-linear controls. Control variable takes a linear time variation until a certain instant of time when it jumps to a different linear variation.

3. Piecewise-linear continuous controls. Similar from piecewise-linear controls with the difference that the control variable is constant over time.

4. Polynomial controls. Control variable vary smoothly over time.

Optimization problems are very common in bioprocessing such as maximize the yield of a reaction or maximize the concentration of a desired product.

Optimization problems can be sorted into two groups.

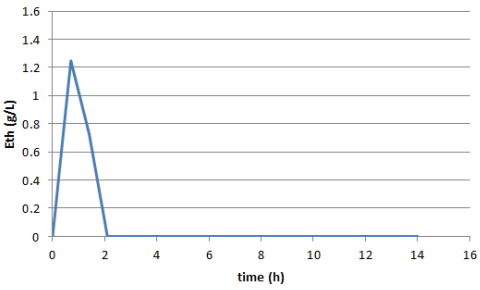
1. Static optimization: it consists in minimizing or maximizining an objective function only in one instant of time.

2. Dynamic optimization: or optimal control: it is referred to the process of maximizing or minimizing an objective function over a known period of time [19].

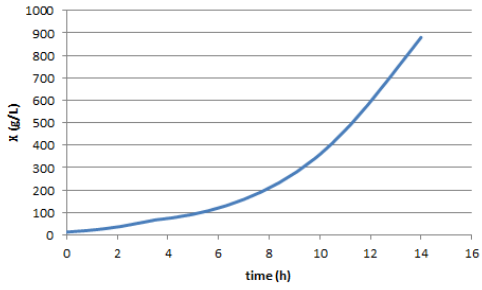
The aim of the optimization is to minimize the production of ethanol while μ. The objectives functions are described by

$$\begin{aligned} &\max_{\dot{V}} \int Eth - \int \mu \\ &\text{Subject to:} \\ &\dot{V} < 0.5. \end{aligned} \tag{3.26}$$

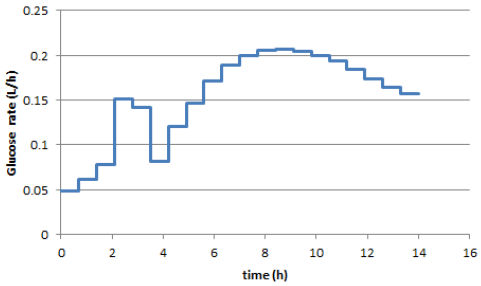
The control variable is the glucose rate and the aeration rate is kept at its maximun, 900 L/h. It is assumed a piecewise-constant control variable. The results of the optimization are shown in Figure 3.13.



(a) Ethanol concentration optimized



(b) Biomass concentration optimized



(c) Glucose optimized

Figure 3.13: Optimization results

There is only one hill caused by Crabtree effect. The production of ethanol has been minimized compared to the results in figure 3.11(d).

The intervals of time at which the control variable changes is 0.7 h. This interval gave the best result, for testing different time intervals.

Chapter 4

Summary

The goal of this thesis was to describe the production of ethanol caused by air limitation during the production process of *Saccharomyces cerevisiae*. Due to the low concentration of oxygen in the reactor ethanol is produced. The oxygen distribution is described by assuming a certain number of phases inside the reactor. The model were simulated in different scenarios. Firstly the model is simulated without the oxygen distribution and with parameters coming from a previous master thesis. The oxygen distribution is introduced in the model for the simulation assuming that the number of phases are 20. Finally, the parameters which came from a previous master thesis are estimated newly. The experimental data which is used to validate the model is derived by VH Berlin. With the new parameters and the oxygen distribution the model is tested again and the ethanol production is achieved to be represented . At the end of the thesis, dynamic optimization is applied. The aim of thes optimization is to obtain as much biomass as possible while minimizing the ethanol. The control variable in the optimization is the feed rate of molasses.

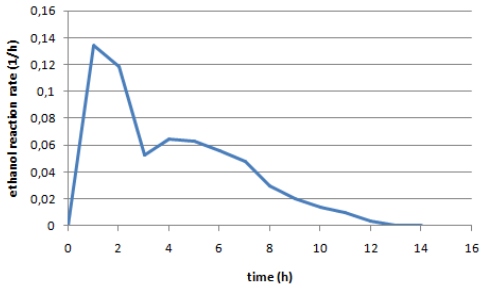
Outlook

As future work, more experiments with different aeration rate and glucose rate can be done. This will provide more information about the process that can be used can be used to validate the current model or to develop it if it is not be able to fit the data new parameter estimation can be done

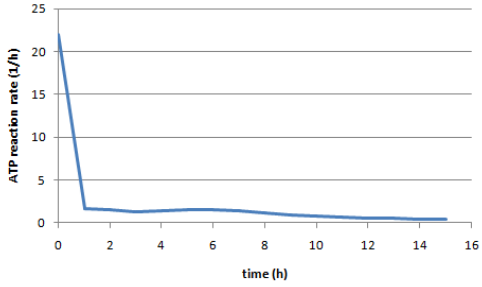
In the industrial process, when the ethanol concentration is higher that a tolerate value, the final product can not be sold. To avoid it, the model can be implemented in a control system that enables to monitor the ethanol concentration over time ethanol. The control system has to be able to detect ethanol production and to reduce it changing the feed rate of molasses.

Appendix A

Simulation results

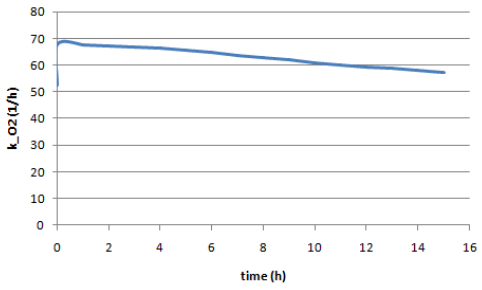


(a) Ethanol consumpiton reaction rate

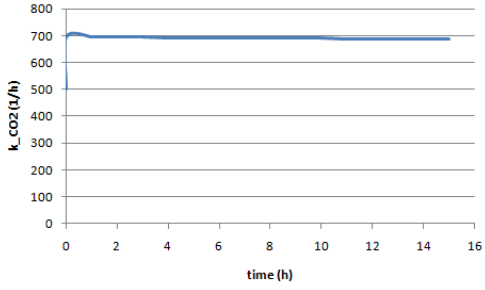


(b) ATP reaction rate

Figure A.1: Reaction rates

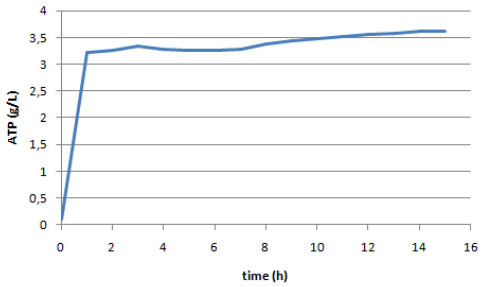


(a) Mass transfer coeficient for the molecules of O_2

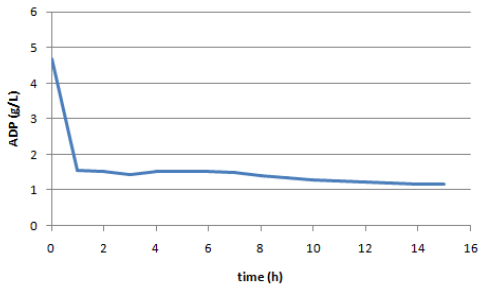


(b) [Mass transfer coeficient for the molecules of CO_2

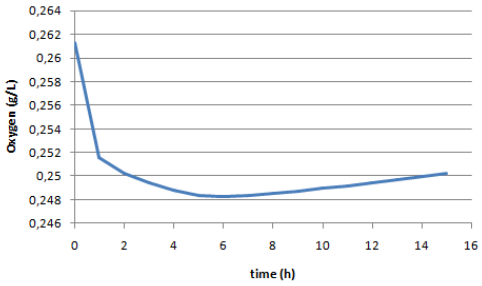
Figure A.2: Mass transfer coefficients



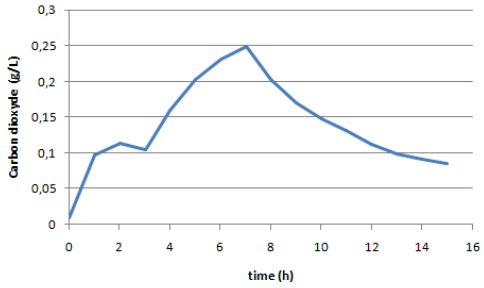
(a) ATP concentration



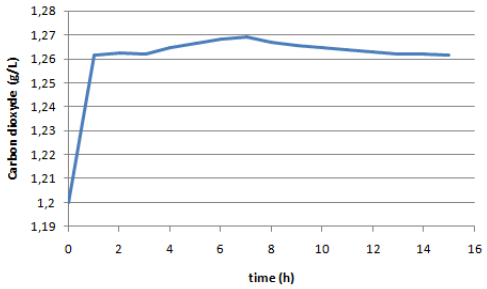
(b) ADP concentration



(c) Concentration of oxygen in the outlet gas flow

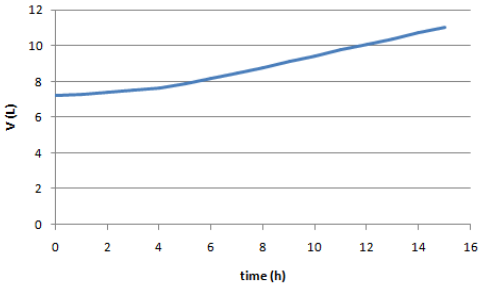


(d) Concentration of carbon dioxygen in the outlet gas flow

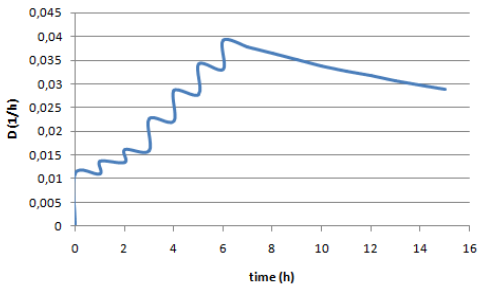


(e) Concentration of carbon dioxygen in the culture

Figure A.3: Metabolites concentration

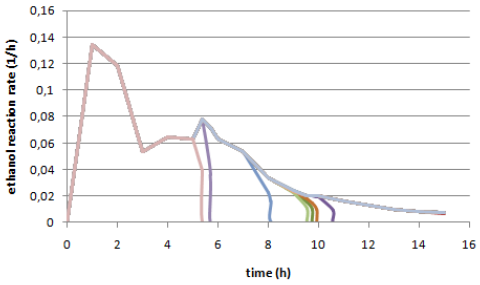


(a) [Volume of the culture

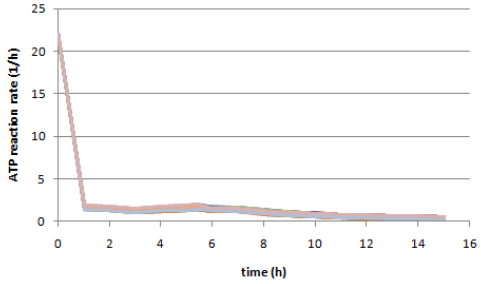


(b) [Dilution rate

Figure A.4: Volume properties

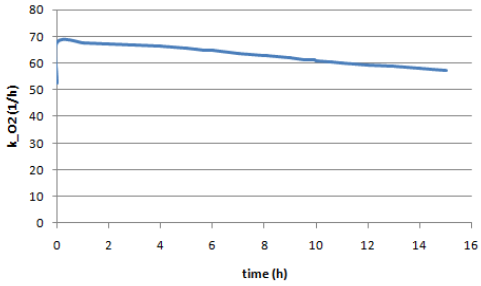


(a) Ethanol consumpiton reaction rate

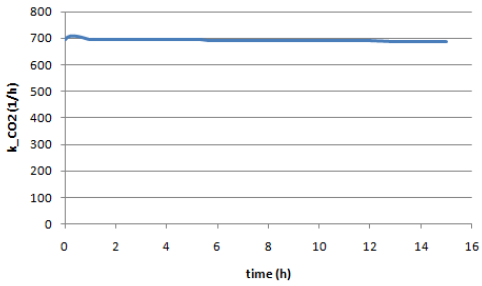


(b) ATP reaction rate

Figure A.5: Reaction rates

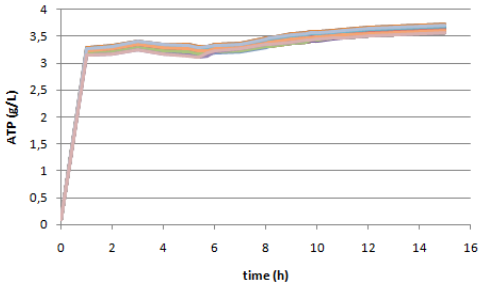


(a) Mass transfer coeficient for the molecules of O_2

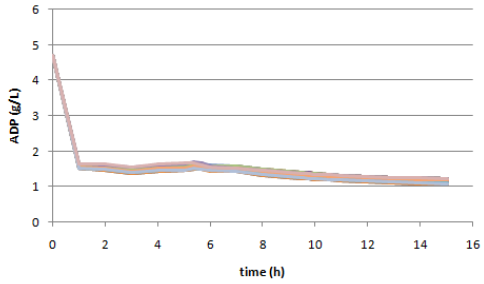


(b) [Mass transfer coeficient for the molecules of CO_2

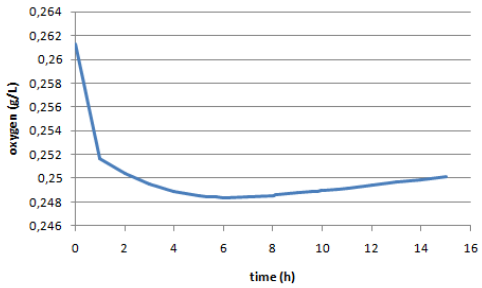
Figure A.6: Mass transfer coefficients



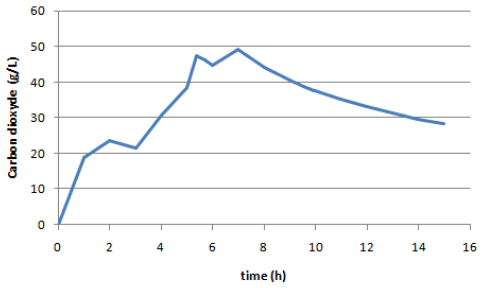
(a) ATP concentration



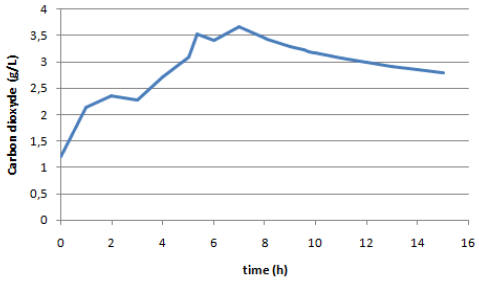
(b) ADP concentration



(c) Concentration of oxygen in the outlet gas flow

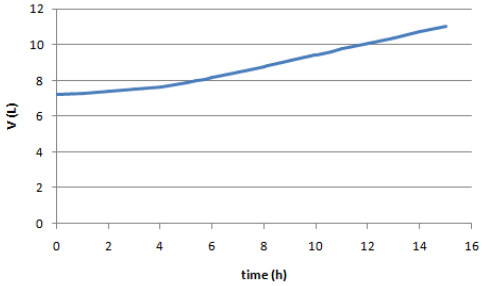


(d) Concentration of carbon dioxygen in the outlet gas flow

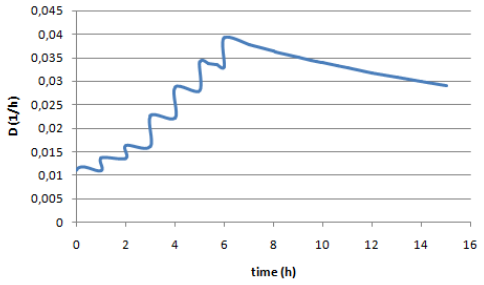


(e) Concentration of carbon dioxygen in the culture

Figure A.7: Metabolites concentration

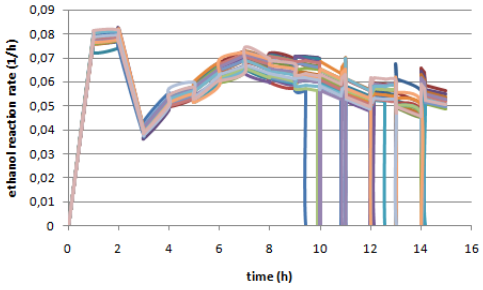


(a) [Volume of the culture

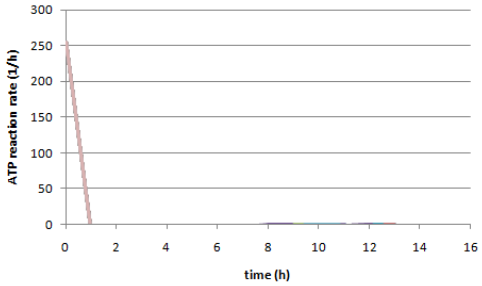


(b) [Dilution rate

Figure A.8: Volume properties

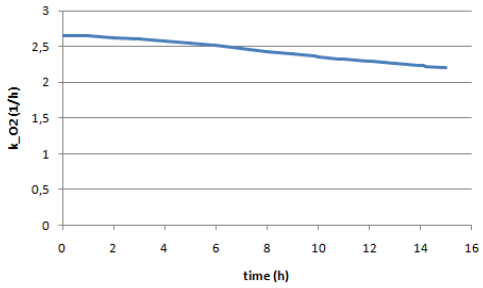


(a) Ethanol consumpiton reaction rate

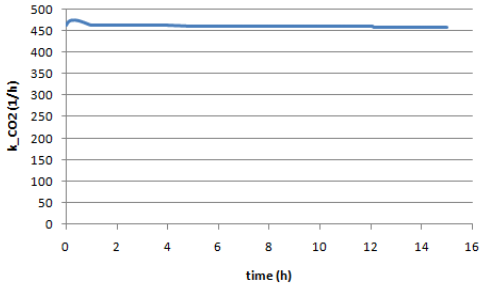


(b) ATP reaction rate

Figure A.9: Reaction rates

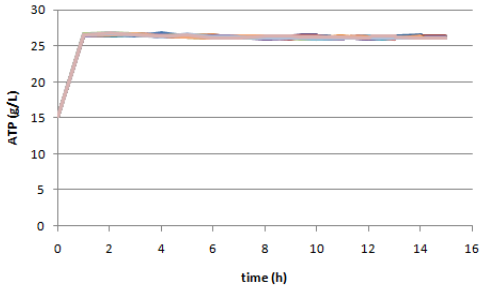


(a) Mass transfer coeficient for the molecules of O_2

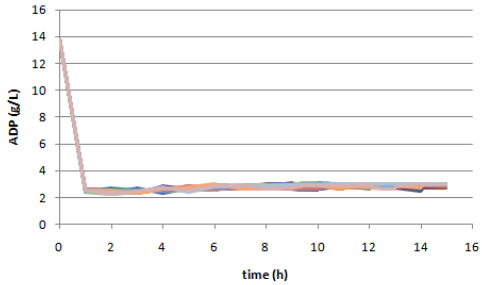


(b) [Mass transfer coeficient for the molecules of CO_2

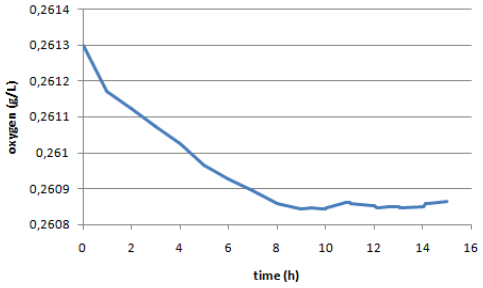
Figure A.10: Mass transfer coefficients



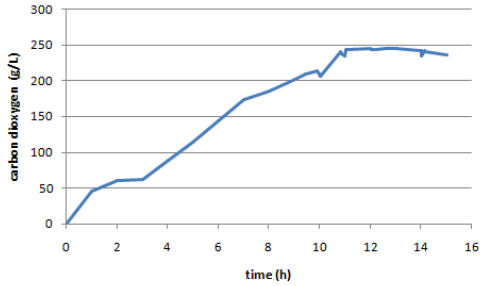
(a) ATP concentration



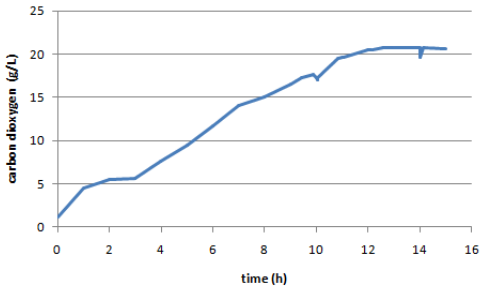
(b) ADP concentration



(c) Concentration of oxygen in the outlet gas flow

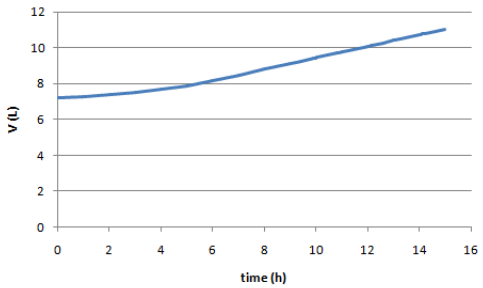


(d) Concentration of carbon dioxygen in the outlet gas flow

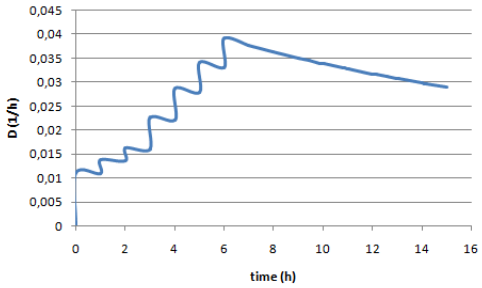


(e) Concentration of carbon dioxygen in the culture

Figure A.11: Metabolites concentration



(a) [Volume of the culture



(b) [Dilution rate

Figure A.12: Volume properties

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