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Development of a Large-Scale Bioprocess Simulation Tool: Engineering and Market Analysis

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KURZFASSUNG

Die computergestützte Simulation von Bioreaktoren bietet eine kostengünstige Alternative zu Laborversuchen, die notwendig sind um Einblicke in ihr Prozessverhalten zu erlangen. In dieser Arbeit wird ein bestehendes Computational Fluid Dynamics (CFD) Simulations-Tool für begaste und gerührte Bioreaktoren, welches auf der Lattice-Boltzmann-Methode beruht, hinsichtlich seiner Marktfähigkeit bewertet und mit biologischen Modellen erweitert.

Hierzu werden Strategien, die sich aus einer Marktanalyse, Konkurrenzanalyse sowie einer SWOT-Analyse des Tools ableiten lassen, in einem Geschäftsmodel und in einer Marketing Strategie umgesetzt. Alleinstellungsmerkmal des Tools ist die schnelle Simulation von großtechnischen Bioreaktoren, welche auch in Zukunft noch von großer Bedeutung sein werden. Die höchste Marktfähigkeit erzielt das Tool mithilfe eines Ingenieurdienstleisters, welcher Bioprozesslösungen anbietet.

Zusätzlich werden zwei nicht segregierte biologische Modelle basierend auf einem nicht strukturierten Ansatz (Herstellung von Gluconsäure) und einem strukturierten Ansatz (Herstellung von Backhefe) implementiert. Die einfachen biologischen Modelle und das Strömungsfeld werden mithilfe eines Euler-Lagrange Ansatzes verknüpft, in welchem Mikroorganismen durch masselose Pellets modelliert werden. Das zu Grunde liegende biologische Modell wird dadurch erst aktiviert, wenn sich mindestens ein Pellet in einer Rechenzelle aufhält. Simulationen beider Fermentationen geben die Konzentrationsverläufe der wachsenden Biomasse, welche mit den einfachen biologischen Modellen berechnet wird, mit mittleren absoluten prozentualen Fehlern von 4.0% (Gluconsäure) und 0.5% (Backhefe) wieder. Der Euler-Lagrange Ansatz ermöglicht die Berücksichtigung von zellinternen Wechselwirkungen sowie das Erfassen der simulierten Produktionsrate einzelner Mikroorganismen.

ABSTRACT

Simulations of bioreactors represent a cheap alternative to laboratory experiments, which are necessary to obtain knowledge about their process behaviour. In this thesis, the marketability of an existing computational fluid dynamics (CFD) simulation tool for aerated and stirred bioreactors based on the lattice-Boltzmann Method is rated and new biological models are implemented.

Strategies derived from a market analysis and a SWOT-analysis are applied in a business model and a marketing strategy. Unique characteristic of the tool is the fast simulation of industrial-scale bioreactors, which will continue to be of great importance in the future. The highest marketability of the tool is achieved with the help of an engineering consulter that offers bioprocess solutions.

Additionally, two non-segregated biological models based on a non-structured approach (production of gluconic acid) and a structured approach (production of yeast) are implemented. To couple the simple biological models with the fluid field, a Euler-Lagrange approach is used where microorganisms are modelled by massless pellets. The underlying biological model is only active if a calculation cell contains at least one pellet. Simulations compared to the biomass concentration trends calculated by the simple biological models result in mean absolute percentage errors of 4.0% (gluconic acid) and 0.5% (yeast). The Euler-Lagrange approach enables a consideration of intracellular activities as well as the tracking of simulated microorganisms' production rates.

DANKSAGUNG

Da sich mit der Erstellung dieser Arbeit ein Lebensabschnitt dem Ende zu neigt, ist es nun an der Zeit, Danke zu sagen.

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1 Introduction

During the last years, the importance of bioprocess technology has significantly increased in different kinds of industries. The heart of bioprocesses are bioreactors, using biological reactions to produce a wide range of products. Production rates in bioreactors depend on physical phenomena like turbulences, mass transfer, biokinetics or fluid dynamics. In order to obtain reliable information about process behaviour of bioreactors, precise models or laboratory experiments are necessary. Due to the now available computational power, computational fluid dynamic (CFD) simulations provide a state-of-the-art method to accurately simulate bioprocesses.

Since the beginning of a multi-annual science collaboration with a leading pharmaceutical company, the Institute of Process and Particle Engineering of the TU Graz is developing a CFD tool for industrial-scale fed-batch bioreactors. This simulation tool is based on a *C++* code, extended with an *CUDA* library for the parallelization of the occurring computational tasks on *NVIDIA* graphic processing units (GPU). The whole simulation tool is developed and optimized for *LINUX* operating systems. Post processing is carried out with the data-analyse and visualization tool *PARAVIEW*.

The developed tool provides a powerful way to model industrial-scale bioreactors and is unique in its modelling approaches. In the course of this thesis, the marketability of the tool should be rated. The rating is based on a market analysis and a SWOT-analysis of the tool. In further consequence, the development of a business model based on the findings of the analyses is carried out. The business model is based on the current most promising selling concept of the tool. Due to the scientific collaboration with a pharmaceutical company, the tool is specialized in this field of application. Therefore, special focus of the research is placed on the pharmaceutical sector. Current capabilities of other available CFD tools were analysed to rate the competitiveness of the developed tool.

Biological models based on different approaches were implemented to increase and demonstrate the tool's capabilities. Furthermore, a coupling of the fluid dynamics with the biological models based on a Euler-Lagrange approach is carried out to consider effects of the fluid field on the applied biological models. The second aim of this thesis is the simulation of whole bioprocesses based on the new extensions of the tool.

The motivation to analyse the marketability of the developed bioprocess simulation tool is directly linked with the industry's need for CFD simulations due to numerous advantages and potentials in comparison to laboratory experiments. Thus, the most important benefits of modelling bioreactors are provided in the following section.

1.1 Motivation and background

During the last decade, CFD has increasingly been used in the biotechnological industry. Based on this modelling approach, parameters like impeller design and speed, gas rate and tank dimensions or shear damage of microorganisms can be determined [1]. Due to the complexity of bioprocesses, pilot experiments and production tests for successful scale-ups are necessary. Typical test runs cost 40.000-80.000 € and the number of tests ranging from ten to fifteen. Therefore, reducing test runs is very important for facility operators. CFD modelling of processes can reduce the necessary number of test-runs to two or three. Optimizing substrate-to-product conversions and minimizing impurities in the biological reaction are also major applications of the developed CFD tool. The cumulative effect of the listed factors may easily sum up to 25% - 50% of manufacturing costs on average. Therefore, optimization of bioreactors carries a huge potential for cost saving [2]. CFD enables also a deeper process understanding due to the realistic and detailed modelling of bioprocesses. Thus, an increase of the Quality by Design (QbD) paradigm is achieved which is expected to result in a further growth of CFD usage in biotechnology [1].

The in-house developed tool provides several capabilities, specialized on engineering tasks in the biopharmaceutical industry. Table 1-1 shows the most important applications of the tool.

Table 1-1: Typical applications of the in-house developed tool

Applications	Corresponding goals
Scale-up/ scale-down	Reducing piloting and production tests
Reactor optimization	Increase reactor efficiency/ product output
Process transfer	Adaption of processes to maintain product quality
Deviation management/ QbD	Predicting effects of changed operation parameter

As shown, the main usages target at saving costs and increasing the product and process quality, whose can be assumed as very important factors for biotechnological companies. The motivation to rate the tool's marketability is based on these factors. To give more insight into the tools field of application and capabilities, an overview about the reactor geometry is provided. Figure 1 shows an isometric section of a typical bioreactor modelled with the in-house developed tool.

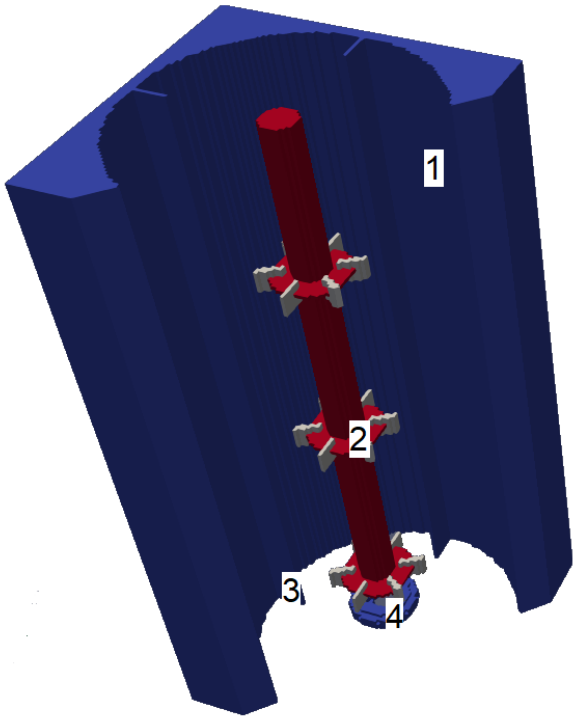

Figure 1: Isometric section of a modelled bioreactor. 1: Reactor wall; 2: Stirrer; 3: Ring sparger for oxygen supply; 4: Mixing baffles

As shown, the basis of the reactor is a cylindrical reactor wall (Number 1) with a variable diameter. The bottom of the reactor can be either flat or with a Klöpper head. To improve mixing, baffles can be considered (Number 4). The oxygen necessary for the biological reactions is provided by air bubbles blown in through a ring sparger, located on the bottom of the reactor (Number 3). Mechanical energy from the stirrer's motor is transferred via the stirrer shaft to the stirrer blades (Number 2). To find the best stirrer setup for a simulation case, stirrers can be adapted in form, number and position. It is also possible to simulate non-vertical stirrers, which are often found in single-use bioreactors. In this thesis, a triple Rushton stirrer is used in the simulations.

A necessary part for a full understanding of the tool is a short introduction into the applied simulation approaches as well as the basics of biological reactions. The fundamentals of them are provided in the next three sections. Rating of the tool's marketability begins in Chapter 5. The idea is to demonstrate the tool's capabilities by modelling and analysing whole bioprocesses, before the economic aspects are rated.

1.2 Modelling fundamentals of the bioprocess simulation tool

The order of magnitude of the physical phenomena in a bioreactor are influenced by the fluid flow field. An accurate and fast modelling of the fluid mechanics is therefore the main challenge of a bioprocess simulation tool. In general, the flow behaviour of a Newtonian fluid inside a stirred bioreactor is described by the Navier-Stokes equations. However, analytical solutions for these equations are only available for basic flows like the Couette or Poiseuille flows [3]. In this tool, the lattice-Boltzmann Method (LBM) is applied on every single calculation cell to approximate the Navier-Stokes equations.

Lattice-Boltzmann Method

In the following, a short introduction into the lattice-Boltzmann Method based on the book "The Lattice Boltzmann Method – Principles and practice" is presented [3]. The LBM is based on the Boltzmann equation that describes dynamics of a gas on a mesoscopic scale as well as fluid dynamics on the macroscale. The mesoscopic scale describes the distribution of particles while the macroscopic scale describes the fluid using quantities like density or velocity.

In order to solve the Boltzmann equation numerically, a discretization in space and time is necessary. In comparison to alternative ways for approximating the Navier-Stokes equations, the LBM requires a constant spatial discretization, which is a major advantage due to simple meshing. Basic quantity of the discretized Boltzmann equation is the discrete-velocity distribution function $f_i(x,t)$ which is also often called *particle population*. This function represents the density of particles with the velocity c_i at position x and time t. Together with a corresponding weighting coefficient w_i, velocity sets $\{c_i,w_i\}$ are formed. In the LBM, these velocity sets are denoted by DdQq. Spatial dimensions are represented by d while q is the number of velocity sets. In the in-house developed tool, a D3Q19 LBM is implemented. Figure 2 shows a D2Q9 and the applied D3Q19 velocity set.

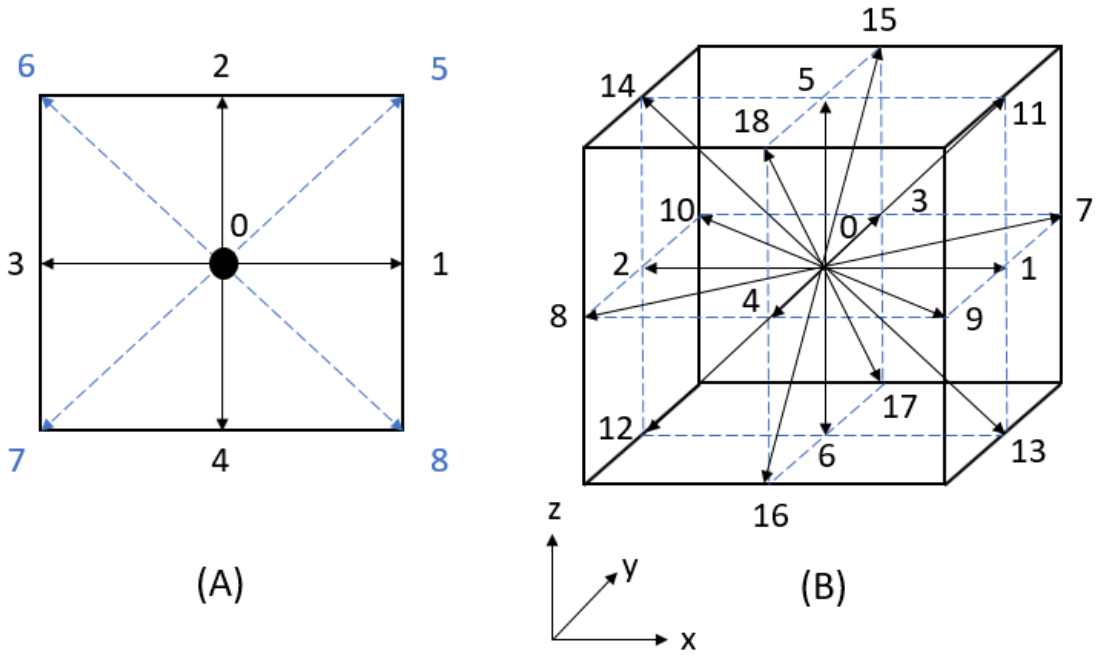

Figure 2: (A): D2Q9 velocity set in a cubic cell. (B): D3Q19 velocity set in a cubic cell. The vector 0 is the rest velocity with zero magnitude to represent stationary particles [3]

Equation 1-1 shows the lattice-Boltzmann equation, which is the Boltzmann equation discretized in velocity space, spatial space and time. The velocity space is described by the number of velocity sets, shown in Figure 2.

$$f_i(x + c_i\Delta t, t + \Delta t) = f_i(x, t) + \Omega_i(x, t) \quad (1\text{-}1)$$

Ω_i represents the collision operator that models particle interactions. The most common one for Navier-Stokes simulations is the Bhatnagar-Gross-Krook collision operator, which is implemented in the in-house developed tool. The equation describes the movement of particles $f_i(x,t)$ with the velocity c_i to the next point $(x+c_i\,\Delta t)$ at the next time step $(t+\Delta t)$. It forms the foundation of the fluid field calculation in the bioprocess simulation tool. Turbulences in the flow field are modelled with the large eddy simulation. A detailed description of the implemented turbulence model can be found in the publication of Witz et al. [4]. Besides the flow field, biological reactions depend on dissolved oxygen from bubbles and the movement of microorganisms in the bioreactor. A Euler-Lagrange approach is used to describe those movements in the tool.

Euler-Lagrange approach

The gaseous air blown in through the sparger forms bubbles moving to the top of the tank due to their buoyancy force. While moving through the fluid filed, mass transfer occurs, and oxygen is dissolved in the fluid. In the in-house developed tool, tracking of the particles in the fluid field is based on a Euler-Lagrange approach. Main advantage of using this method is that the location of the particles is always known. The Euler-Lagrange approach describes the movement of the continuous phase (i.e. liquid) in fixed control volumes (i.e. calculation cells) while the trajectories of the dispersed phase (i.e. bubbles, pellets) are tracked. This means that fixed spots are observed with the Euler approach while the positions of moving pellets or bubbles are followed with the Lagrange approach. Forces on a bubble or pellet leads to a change of the trajectory. Figure 3 shows a schematic explanation of the Lagrange approach for the disperse phase.

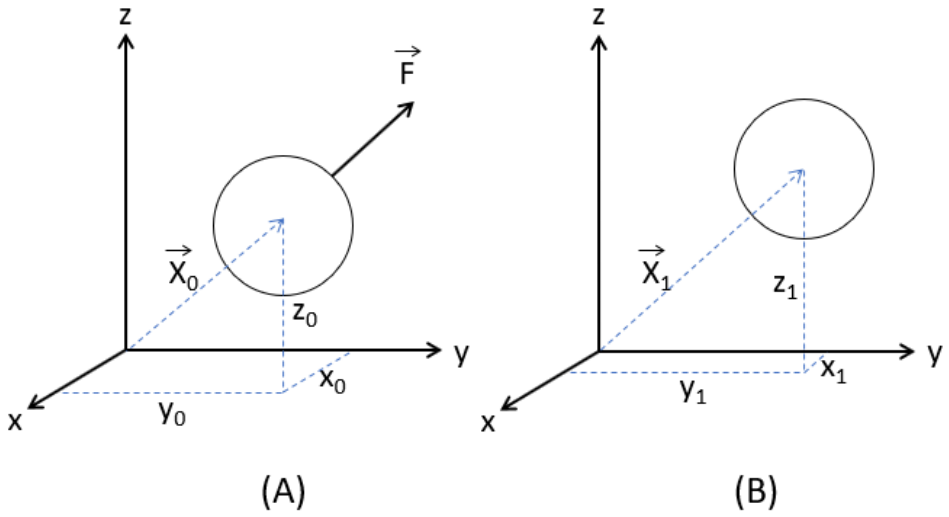

Figure 3: Lagrange approach for bubble movement. (A) represents the bubble at time step 0, (B) at time step 1. The spatial vector X consists of the three coordinates x,y,z. F represents a force at time step 0

Figure 3 shows the change of the bubbles position from time step zero to time step one. The difference to the Euler method is that the position in space is always known.

In the in-house developed CFD tool, microorganisms are considered as mass and volume free pellets due to their negligible small size. These pellets are also modelled with the Euler-Lagrange approach. In comparison to the bubble modelling, mass forces are not considered. Therefore, flow behaviour of the pellets depends completely on the fluid flow field.

For modelling a full multi-phase bioreactor, turbulences as well as bubble behaviour like coalescence and break-up also needs to be considered. However, focus of the tool's extension lies on the implementation and application of biological models. More information about the applied modelling approaches as well as alternative approaches can be found in the publication of Witz et al. and are not further explained in this thesis. Besides the presented physical phenomena, biokinetics represent the second pillar for modelling full bioprocesses and are explained in the next section.

1.3 Fundamentals of biologic reactions

In general, biotechnological manufacturing utilizes biological reactions (often called fermentations) to produce a certain product by the mass culture of a microorganism [5]. Industrial fermentation can be separated into five major groups, depending on their products. Table 1-2 shows the five groups and typical kind of products as well as their application and corresponding industry.

Table 1-2: General fermentation processes separated into their products [5]

General products	Example products	Industries	Application
Microbial cells (or biomass)	Yeast	Food	Baking
Microbial enzymes	Amylase	Baking and milking	Reduce mixing time
	Amylase, protease	Pharmaceutical	Digestive aids
Microbial metabolites	*Primary products of microbial metabolism*		
	Ethanol	Food	Alcoholic drinks
	Gluconic acid	Pharmaceutical	Calcium therapy
	Secondary products of microbial metabolism		
	Penicillin	Pharmaceutical	Antibiotics
Recombinant products	Insulin	Pharmaceutical	Hormone
Modification of a compound that is added to the fermentation (Transformation process)	Vinegar	Food	Vinegar
	Steroids	Pharmaceutical	Hormones
	Antibiotics (Mammalian cells)		Antibiotics

The table shows the large variety of products made by fermentations. For detailed information about the presented fermentation types, [5] is highly recommended. In order to model fermentations in the bioprocess simulation tool, mathematical models of biological reactions are necessary.

During a fermentation, growth of the microorganisms and enzymatic or microbial reactions occur simultaneously. One simple relationship to describe the general observations occurring on a growing cell in a biological system is the Monod equation. It presents a highly simplified model of the numerous and complex biological reactions occurring in a real cell. To show the occurring reactions in a biological system, the Michaelis-Menten equation is derived. It shows the same basic form as the Monod equation and enables a fundamental understanding of biological systems, because all fermentations are controlled by enzymes [6].

Michaelis-Menten equation

The Michaelis-Menten equation describes most reactions catalysed by soluble enzymes. It's underlying mechanism shows similarities to the reaction mechanism of heterogenous catalysis as shown in Equation 1-2 and Equation 1-3. The following derivation is based on the book "Biological Reaction Engineering" [6].

$$S + E \overset{k_{-1}}{\underset{k_1}{\longleftrightarrow}} ES \tag{1-2}$$

$$ES \xrightarrow{k_2} P + E \tag{1-3}$$

In these equations, the substrate is represented by index *S*, the enzyme by index *E*. The rate constant k_1 represents the forward reaction, k_{-1} the backward reaction and k_2 the reaction of the enzyme-substrate complex *ES* to a product *P*. The substrate is the reactant of a biological reaction while the enzyme is a catalysator which processes the substrate without being used. The enzyme-substrate complex can be seen as a temporary product of the enzyme and is further processed to a product *P*. The enzymes produced *P* are now available for new conversions of substrate. Equation 1-2 and Equation 1-3 can also be written in differential form for batch reactions, as shown in Equations 1-4 and 1-5.

$$\frac{dC_S}{dt} = -k_1 * C_S * C_E + k_{-1} * C_{ES} \tag{1-4}$$

$$\frac{dC_{ES}}{dt} = k_1 * C_S * C_E - (k_{-1} + k_2) * C_{ES} \quad (1\text{-}5)$$

Schematic concentration changes based on these equations are shown in Figure 4.

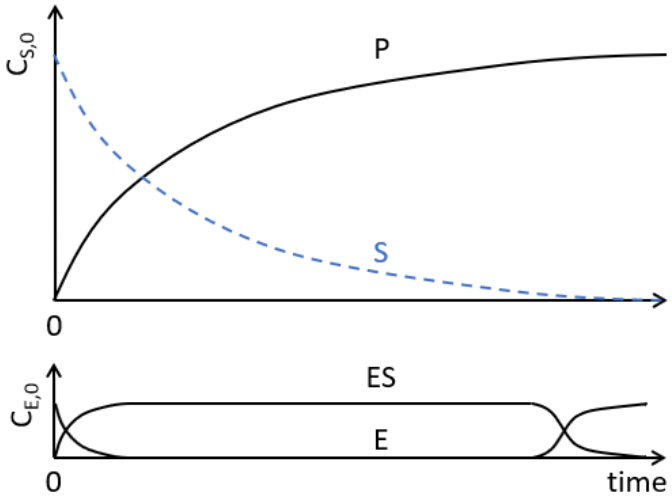

Figure 4: Schematic time trend of substrate *S*, product *P*, enzyme *E* and enzyme-substrate *ES* concentrations of a simple Michaelis-Menten kinetic model [6]

By assuming a batch reaction, the initial conditions at reaction time t=0 can be set with C_S=$C_{S,0}$ and C_{ES}=0. Based on the linear ES trend, a quasi-steady state for the substance can be assumed as shown in Equation 1-6.

$$\frac{dC_{ES}}{dt} = 0 \quad (1\text{-}6)$$

Necessary constraint for this assumption is that E<<S_0. Based on the presented assumptions, the systems substrate balance equation is shown in Equation 1-7.

$$-\frac{dC_S}{dt} = \frac{k_2 * C_S * C_{S,0}}{\frac{k_{-1} + k_2}{k_1} + C_S} \quad (1\text{-}7)$$

By combining some terms, the Michaelis-Menten equation can now be formed as shown in Equation 1-8.

$$r_S = \frac{v_m * C_S}{K_M + C_S} \quad (1\text{-}8)$$

The maximum reaction rate v_m and the Michaelis-Menten constant K_M summarize terms of Equation 1-7. However, to describe simple growth kinetics of the biomass itself, the Monod equation is necessary.

Monod equation

In a batch fermentation with the perfect environment for the microorganism, growth of biomass is almost exponentially with respect to time. The reason for this phenomenon is that all cells have the same probability to multiply. Thus, a first order rate expression describes the growth kinetics, as shown in Equation 1-9.

$$r_X = \frac{dC_X}{dt} = k * C_X \tag{1-9}$$

In this equation, r_x represents the rate of cell growth, C_X the biomass concentration, and k is a kinetic growth constant. Equation 1-10 presents the analytical solution of this equation, with $C_{X,0}$ as the cell concentration at time t= 0.

$$\frac{C_X}{C_{X,0}} = e^{k*t} \tag{1-10}$$

Plotting this equation over the natural logarithmic cell concentration and time results in a linear growth curve. Figure 5 shows the schematic growth behaviour of a cell in a biological system.

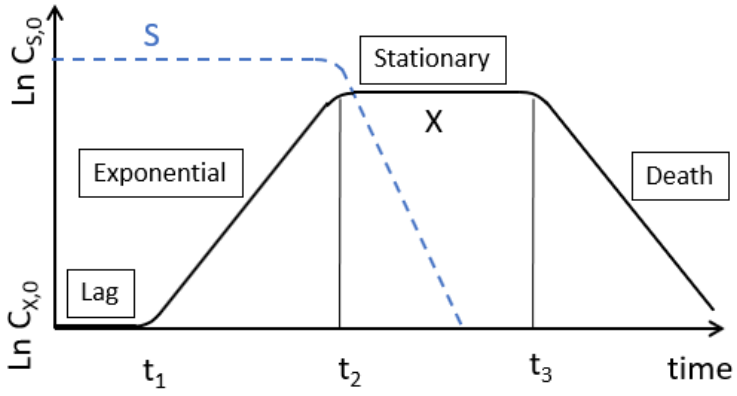

Figure 5: Schematic time trend of logarithmic biomass *X* and substrate *S* concentrations, described with a Monod-equation [6]

A cell goes through different stages in its lifetime, as shown in the figure. At first, the cell has to adapt itself to its environment in the lag phase before t_1. After its exponential growth, substrate limitation occurs and the cell growth stops after reaching the stationary phase. After a certain lifetime, cell death occurs. The slope of the linear growth between t_1 and t_2 is described with the constant k. In the literature, this kinetic growth constant is called specific growth constant with the abbreviation μ. In similar form to the Michaelis-

Menten equation, the exponential and limitation phase can be described with the Monod equation, shown in Equation 1-11

$$\mu = \frac{\mu_m * C_S}{K_S + C_S} \tag{1-11}$$

The maximum growth rate is limited through μ_m and K_S represents a saturation constant for substrate uptake of the cell. The maximum growth rate μ_m is achieved when C_S is close to infinity.

Different kinds of models to describe a whole biological system can be found in literature, which are discussed in the next section. These models are based on the presented Monod- and Michaelis-Menten equation.

Mechanistic models of biological systems

Similar to chemical reactions, the dynamics of biological reactions can either be described with mechanistic or empirical models. Mechanistic models are mathematical descriptions of internal processes of a system. For example, mechanisms in a biological system can be microbial growth, oxygen uptake, and production rate. Thus, the mechanisms are determined by constituents of the biological system like substrate, oxygen or biomass [7]. In this thesis, the focus lies on mechanistic models because they are based on mass and energy balances. Furthermore, empirical models are only valid for the range of data used for developing the model [7]. Mechanistic models of cell populations can be classified into four different types, as shown in Figure 6.

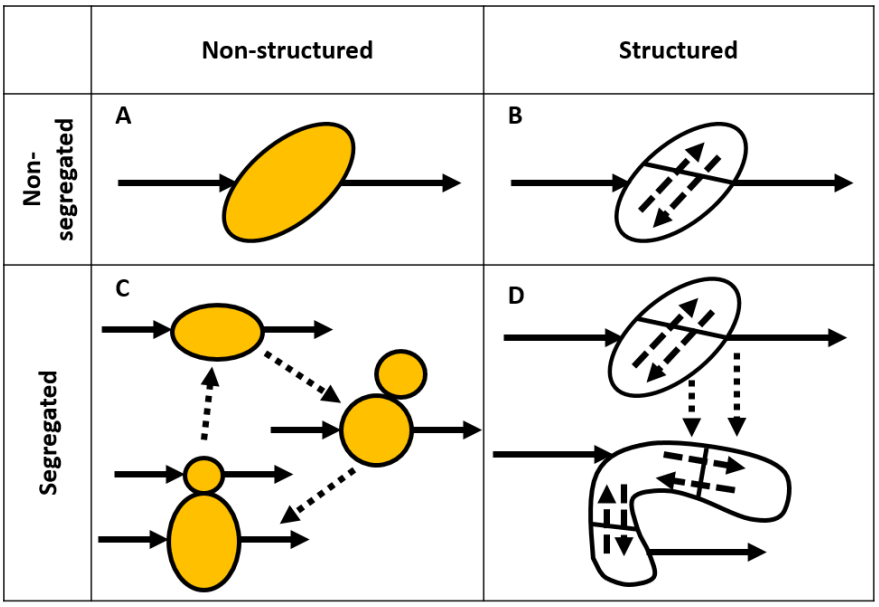

Figure 6: Overview of mechanistic models for the description of biologic systems [6]

Mechanistic models can be categorized into non-segregated and segregated as well as non-structured and structured models. The first category, non-segregated models, are the simplest one because they are based on an average cell description. It's subcategory, non-structured models, describe biomass with one single variable. On the other side, structured models describe the biomass with several variables because of the detailed consideration of intracellular interactions. Thus, more complex bioprocesses can be described with this method like the growth of yeast including its intracellular metabolism. Segregated models consider the fact that cells, occurring always in a population, differ from each other. Extracellular interactions can be considered with this approach. To model the differences in cells of a mass culture, population balance models are often used. Same as for the non-segregated models, structured and non-structured approaches exist. While non-structured segregated models describe cells by one distributed property like cell size or age, structured models consider several distributed intracellular properties [7]. Lapin et al. showed, that this concept can be applied to model a *E.Coli.* population in a bioreactor based on CFD [8]. In this publication, bacteria cells are modelled with a Lagrange approach. Due to the limited resources in the early 2000s, bacteria cell movement is determined with stochastic equations (Population balance equations) and its calculation is performed separated to the fluid field. However, this work presents a

significant improvement of the Euler-Euler approach in bioreactor modelling, because interactions of the cell population can now be considered.

Haringa et al. presents a more recent work based on a Euler-Lagrange approach [9]. In this publication, the fermentation of *Penicilium chrysogenum* is modelled with a simplified modelling approach to minimize computational effort. For example, no gaseous phase is considered, and fluid dynamics and the biological process are calculated separately. However, it uses a Euler-Lagrange approach without population balance equations. The cell population is modelled by a large number of spheres that are larger than several calculation cells. It allows them to consider substrate concentration gradients and substrate transfer inside the spheres and thus, a consideration of a non-instantaneous adaption of the modelled microorganisms to their surroundings [9].

Because the application of a population balance model demands high computational effort, the approach of Haringa et al. for the implementation of a biological model is followed. The full approach can only be applied for simplified biological models that depend only on substrate, because the underlying artificial parameters that describe the intracellular activities are only available for this reactant. Thus, spheres smaller than a calculation cell (pellets) without the consideration of intracellular substrate gradients are used. Detailed information about the implemented approach and the biological models are presented in the next chapter.

2 Materials and methods

For an effective extension of the bioprocess simulation tool, the selection of the biological models as well as the coupling of the biokinetics to the fluid field is of major importance. Thus, two different kinds of validated biological models with different approaches are chosen for implementation. Both are compared in literature with real lab-scale bioprocesses and show accurate results. Furthermore, a Euler-Lagrange based approach for the biological model is used. It sets the rules for the coupling of the fluid dynamics and biological models and is explained in the following section.

2.1 Coupling fluid mechanics with biokinetics

As mentioned in the last chapter, mass free pellets, modelled with a Lagrange approach are already considered in the in-house developed tool. With these pellets, flow behaviour as well as mechanical stress of bacteria cells in a bioreactor can be observed and analysed.

However, depending on the complexity of a biological reaction, a varying number of reaction participants are necessary to carry out biological reactions in the bioreactor. As already explained in Section 1.3, the product concentration of a bioprocess depends on known concentrations of the reaction participants. Reaction participants in this thesis are in general substrate, biomass and oxygen as well as intracellular parameters for the yeast fermentation. These participants as well as the reaction products itself are influenced by the occurring fluid field. Fluid mechanics, mass transport and biological models are calculated in equally distributed cubic nodes with a resolution of 179 nodes per meter, which is equal to an edge length of about 5.6 mm. Moving pellets with the diameter 0.001 mm are smaller than the calculation nodes and calculated several times during one fluid time step. The coupling of the biological model with the fluid field is based on activating the biological model in calculation cells with pellet contact, as shown in Figure 7.

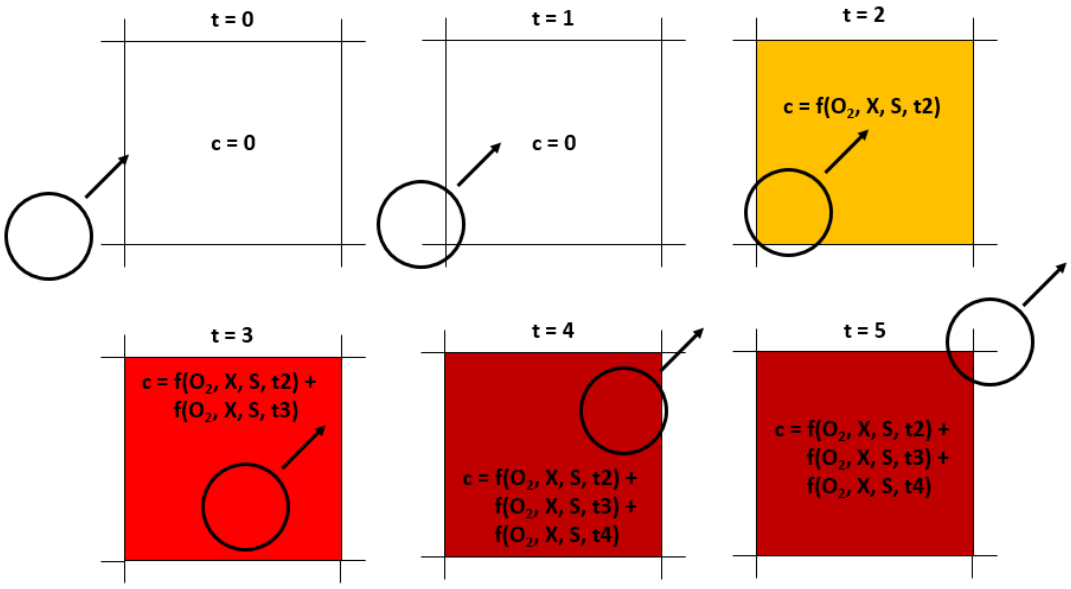

Figure 7: Applied method to couple fluid dynamics and biological models with mass-less pellets in the bioprocess simulation tool. Product concentration in a calculation cell depends on oxygen, biomass, substrate and time steps

As shown in the figure, the calculation cell does not contain a pellet at time t=0. At t=1, the pellet enters the cell, but not with its centre. With entering the cell with its centre, the pellet activates the biological model at t=2. For every time step the pellet remains with its centre in the node, the biological model is active. Reaction participants needed for the biological model decreases while product and biomass increase due to the reaction. After leaving the calculation node with the pellets centre, the biological model for the considered cell is deactivated and no further reaction occurs, as represented with time step t=5. If more than one pellet is in one calculation cell, all reaction participants are divided through the number of pellets in the cell. One major advantage of this approach is the recorded lifetime of every pellet, as shown in [9]. Additionally, intracellular activities can be modelled. Production rate over time and space can be analysed and used for optimization tasks on the bioreactor, as shown later in Chapter 4. The theoretical frameworks of the implemented biological models are explained in the following two sections.

2.2 Unstructured and non-segregated biological model: Gluconic acid

The first model represents the aerobic biological production of gluconic acid through an *Aspergillus niger* strain and is based on the Monod equation [10]. Gluconic acid is a common product in food industry (stabilizer) as well as in the pharmaceutical industry (iron gluconate). A two-phase Euler-Euler CFD simulation with this biological model is already carried out in a 5 L bioreactor and validated with experimental data [11]. The modelled reaction needs glucose and oxygen to produce gluconic acid and the growth of the biomass, *A. niger*. It is based on four first order, linear ordinary differential equations, as shown in Equation 2-1, 2-2, 2-3 and 2-4.

$$r_X = \frac{dC_X}{dt} = \mu * C_X \tag{2-1}$$

$$r_S = \frac{dC_S}{dt} = -\gamma * \frac{dC_X}{dt} - \lambda * C_X \tag{2-2}$$

$$r_{O_d} = \frac{dC_{O_d}}{dt} = K_L a * (C_{O_d}{}^* - C_{O_d}) - \delta * \frac{dC_X}{dt} - \varphi * C_X \tag{2-3}$$

$$r_P = \frac{dC_P}{dt} = \alpha * \frac{dC_X}{dt} + \beta * C_X \tag{2-4}$$

The specific growth parameter μ is shown in Equation 2-5.

$$\mu = \mu_m * \frac{C_S}{K_S + C_S} * \frac{C_{O_d}}{K_O + C_{O_d}} \tag{2-5}$$

The terms r_X, r_S, r_{Od} and r_P are the rates of biomass growth, substrate and oxygen uptake and gluconic acid production. The according concentrations in kg/m³ are represented with C and time is shown with t. K_S, K_O, α, β, γ, δ, λ, μ_m and φ are rate constants, describing cell growth, product generation and substrate as well as oxygen uptake. Equation 2-3 contains the term of the oxygen transfer rate (OTR), consisting of the volumetric mass transfer coefficient K_La, the dissolved oxygen concentration C_{Od} and the dissolved oxygen saturation concentration $C_{Od}{}^*$. This term is also provided by the tool.

Corresponding concentration curves of the four components over 60 h fermentation are shown in Figure 8. Initial value for the substrate concentration is 180 kg/m³, 0.01 kg/m³ for the biomass concentration. Same as in the publication, a constant K_La value with 0.017 s^{-1} is set. The dissolved oxygen saturation concentration is set with 0.006 kg/m³. The applied solution strategy for the set of differential equations is explained in Section 2.4. Values of applied parameters are shown in the appendix at Section 10.3.

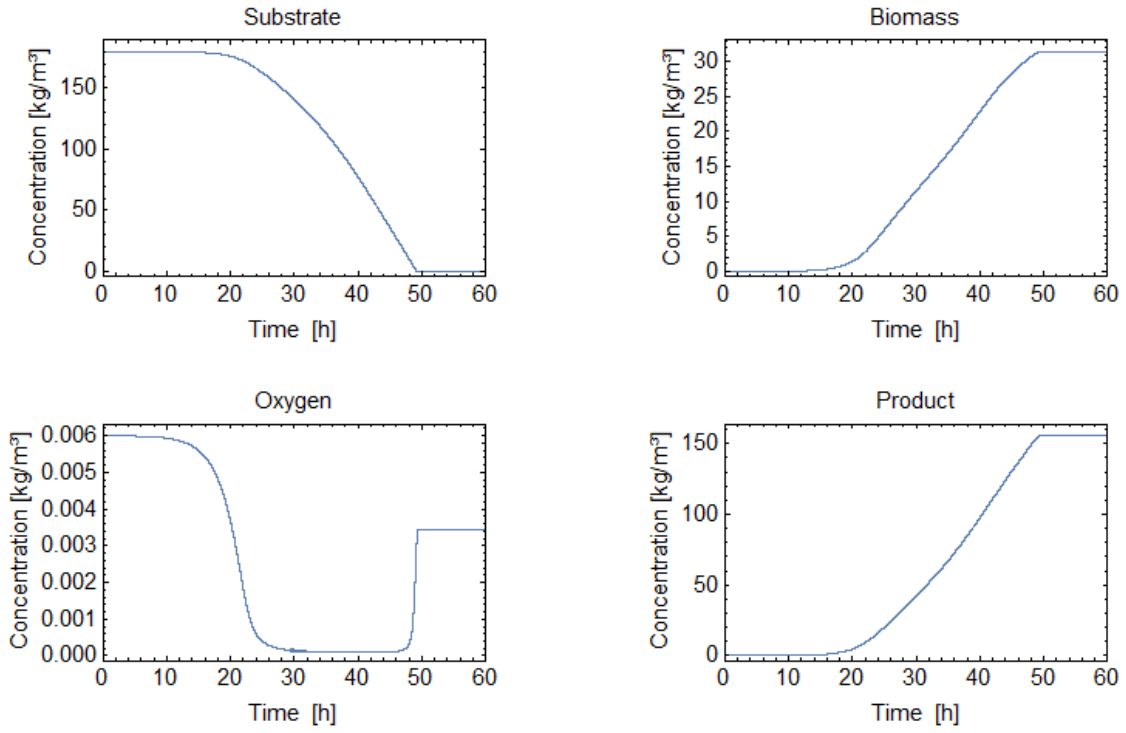

Figure 8: Substrate (glucose), biomass (*Aspergillus niger*), oxygen and product (gluconic acid) development in a batch fermentation described by an unstructured and non-segregated biological model for the production of gluconic acid

As shown in the Figure, substrate and dissolved oxygen are decreasing while biomass and product amount increases. Lag phase is over after 15 h and the exponential biomass growth begins, which also leads to an increase of produced gluconic acid. After 30 h, oxygen is almost depleted, and oxygen provided with the K_La term is completely used for biomass growth. Thus, biomass continuous growth although oxygen concentration is almost zero. After the whole substrate is depleted, the biological reaction is stopped and the amount of solved oxygen increases. The oxygen concentration remains constant due to the equilibrium of the provided oxygen and the uptake, depending linear on the biomass concentration. The presented fermentation is calculated based on the biological model. An application of the model in the bioprocess simulation tool with consideration of fluid mechanics and an oxygen field modelled in detail is presented in Chapter 4.1.

2.3 Structured and non-segregated biological model: Yeast

This structured and non-segregated model represents the complex aerobic *Saccharomyces cerevisiae* (yeast) fermentation from glucose. It is based on a cybernetic modelling approach, which means that microorganisms optimize the usage of available substrate [12]. Yeast is mainly used in the chemical industry for ethanol fuel and in the food industry. In this process, the biomass yeast is the product, ethanol a by-product and glucose the substrate. Oxygen is also necessary for the fermentation. The difference to the previous model is the consideration of three different metabolic pathways during the biomass growth, controlled through enzymes. The first one, glucose fermentation, is active and produces ethanol while the substrate in form of glucose is available. Below a glucose concentration of 0.05 kg/m³, the second metabolic pathway, glucose oxidation, is active and yeast consumes the remaining glucose. After the whole glucose is depleted, the third metabolic pathway, ethanol oxidation is activated. In this metabolic process, the produced ethanol is used for yeast growth. After the fermentation is completed, glucose and produced ethanol is depleted and yeast is produced. During the process, a significant amount of carbon dioxide and heat is released.

The presented model is based on six linear ordinary differential equation and a set of additional functions. In the following, the mathematical framework of the model is presented. At first, functions for growth are described. The growth rates r_i for each pathway are presented in following equations. Equation 2-6 represents the glucose fermentation, Equation 2-7 the ethanol oxidation and Equation 2-7 the glucose oxidation, respectively.

$$r_1 = \mu_1 * e_1 * \frac{C_S}{K_1 + C_S} \tag{2-6}$$

$$r_2 = \mu_2 * e_2 * \frac{C_P}{K_2 + C_P} * \frac{C_{O_d}}{K_{O_2} + C_{O_d}} \tag{2-7}$$

$$r_3 = \mu_3 * e_3 * \frac{C_S}{K_3 + C_S} * \frac{C_{O_d}}{K_{O_3} + C_{O_d}} \tag{2-8}$$

Concentrations in kg/m³ of ethanol, glucose and oxygen are represented by C and the corresponding indices P, S and O_d, respectively. K_i and K_{Oi} are saturation constants for

carbon substrate and dissolved oxygen for all pathways. The rate constants μ_i with the unit 1/h are described by the following Equation 2-9.

$$\mu_i = \mu_{i,m} * \frac{\mu_{i,m} + \beta}{\alpha + \alpha^*} \tag{2-9}$$

The maximum specific growth rate for each pathway is given by $\mu_{i,m}$. Enzyme synthesis parameter are represented with α, α^* and β. Key enzyme concentrations in gram per gram cell mass, responsible for the occurrence of the pathways, are represented with e_i and the corresponding differential equation for the growth rate r_{ei} is shown in Equation 2-10. S_i is a placeholder for the substrate of the pathway.

$$r_{e_i} = \frac{de_i}{dt} = \alpha * u_i * \frac{S_i}{K_i + S_i} - \left(\sum_j (r_j * v_j) + \beta\right) * e_i + \alpha^* \tag{2-10}$$

The cybernetic variables u_i and v_i can be found in the appendix in Section 10.2, Equation 10-1 and 10-2. Differential equations for the concentration change in kg/m³ of biomass *X*, glucose *G*, ethanol *P*, oxygen O_d are shown in Equation 2-11, 2-12, 2-13 and 2-14. The change of the intracellular storage carbohydrate mass fraction in gram per gram cell mass is shown in Equation 2-15.

$$r_X = \frac{dC_X}{dt} = \left(\sum_i r_i * v_i\right) * C_X \tag{2-11}$$

$$r_S = \frac{dC_S}{dt} = -\left(\frac{r_1 * v_1}{Y_1} + \frac{r_2 * v_2}{Y_2}\right) * C_X - \phi_4 * \left(C_C * \frac{dC_X}{dt} + \frac{dC_C}{dt}\right) \tag{2-12}$$

$$r_P = \frac{dC_P}{dt} = \left(\phi_1 * \frac{r_1 * v_1}{Y_1} + \frac{r_2 * v_2}{Y_2}\right) * C_X \tag{2-13}$$

$$r_{O_d} = \frac{dC_{O_d}}{dt} = K_L a * \left({C_{O_d}}^* - C_{O_d}\right) - \left(\phi_2 * \frac{r_2 * v_2}{Y_2} + \frac{r_3 * v_3}{Y_3}\right) * C_X \tag{2-14}$$

$$r_C = \frac{dC_C}{dt} = \gamma_3 * r_3 * v_3 - (\gamma_1 * r_1 * v_1 + \gamma_2 * r_2 * v_2) * C_C - \left(\sum_i r_i * v_i\right) C_C \tag{2-15}$$

Y_i, φ_i and γ_i represent yield coefficients, stoichiometric coefficients for the substrates and stoichiometric coefficients for intracellular carbohydrate storage. Equations for the carbon dioxide production with the index CO_2 and the heat production with index *Q* are shown in the appendix in Section 10.2, Equation 10-3 and 10-4. The heat production rate is based on a linear function of the glucose uptake rate, presented in [13].

Concentration profiles for glucose, biomass, oxygen and ethanol, production profiles of carbon dioxide and heat, growth rate trends for each metabolic pathway as well as the trend of the mass fraction for carbohydrate storage are shown in Figure 9.

As shown in Figure 9, the glucose fermentation pathway leads to a decrease of glucose since the beginning of the fermentation while oxygen concentration remains almost constant and biomass grows exponentially after a lag phase at the beginning. After 9 h, the low glucose concentration results in a short decrease of oxygen and a decrease of the glucose fermentation growth rate r_1 until the pathway of the glucose oxidation r_3 takes over and intracellular carbohydrate storage increases drastically. During the short period of time where r_3 becomes larger than r_1 and r_2, oxygen concentration increases and the ethanol oxidation growth rate r_2 increases. After 10 h, the ethanol oxidation begins, and ethanol is used for yeast growth. But at first, intracellular carbohydrate storage is depleted, which leads to a second lag phase in biomass growth. At 17 h, ethanol is completely depleted and the fermentation ends. Heat production takes place during glucose consumption. Carbon dioxide production is directly linked with the growth of yeast. The amount of produced CO_2 and heat refers to the presented reaction in a one m^3 bioreactor.

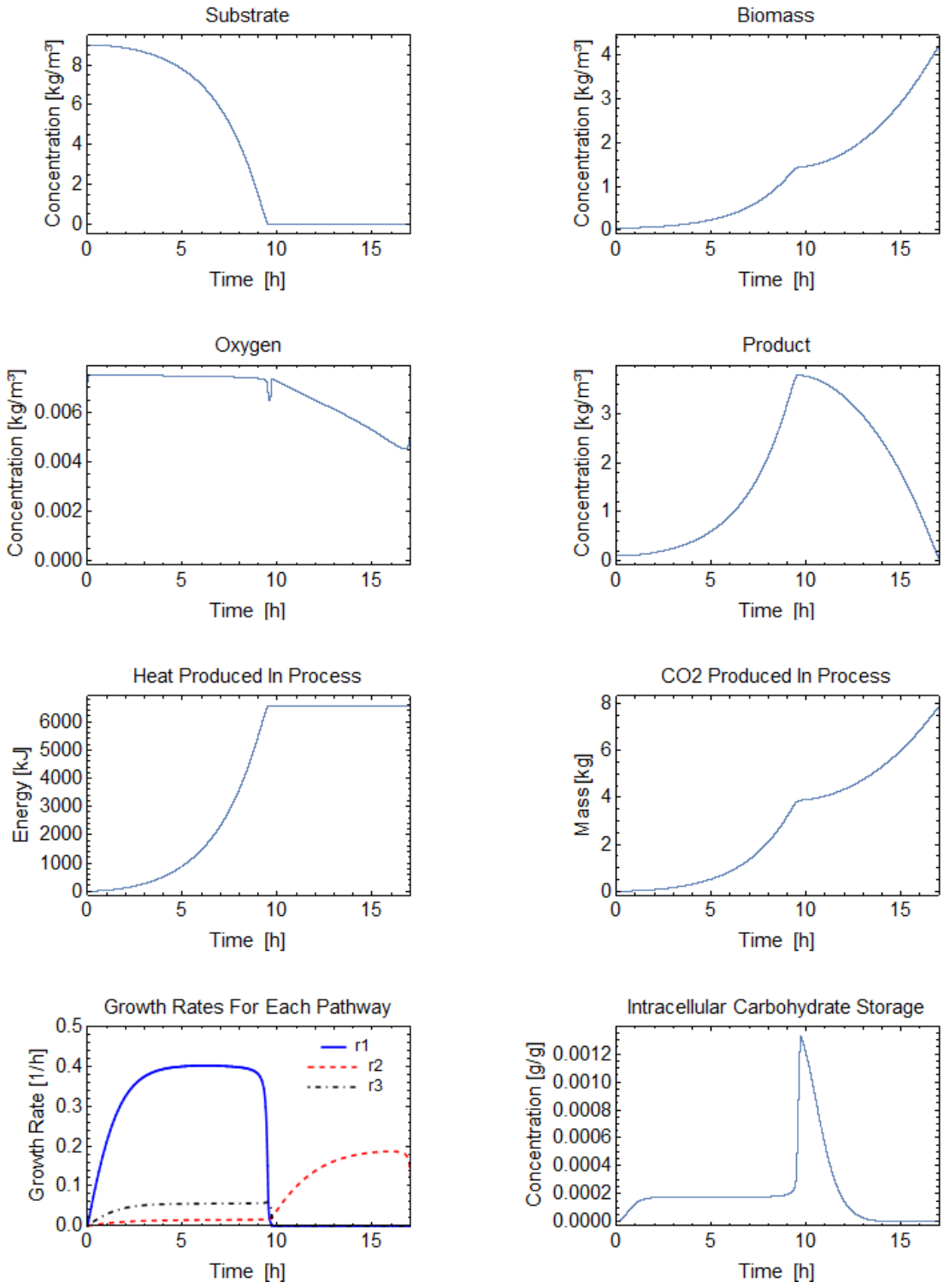

Figure 9: Substrate (glucose), biomass (yeast), oxygen, product (ethanol), overall heat production, carbon dioxide production, growth rates and stored carbohydrate development in a batch fermentation described by a structured and non-segregated biological model for the growth of yeast

The next section provides the implemented solution strategy for the differential equations in both models. Application of the presented model in a bioprocess simulation tool is shown in Section 4.2. Detailed information about values of all applied parameters can be found in the appendix at Section 10.3.

2.4 Solution strategy for ordinary differential equations

As the biological models consist of differential equations, a numerical solving strategy is necessary. Due to the low computational effort, the explicit Euler method for discretization and solving of the equations is chosen [14]. The necessary form of ordinary differential equations is shown in Equation 2-16.

$$\dot{x} = \frac{dx}{dt} = f(t, x) \tag{2-16}$$

Discretization in time is now applied to replace the differential quotient with difference quotients. Final form of the explicit Euler method is shown in Equation 2-17.

$$x^{k+1} = x^k + \Delta t * f(t^k, x^k) \tag{2-17}$$

Based on this equation, the function value of the time step *k+1* depends only in known values, which is easy to calculate. The function principle of the method is shown in Figure 10.

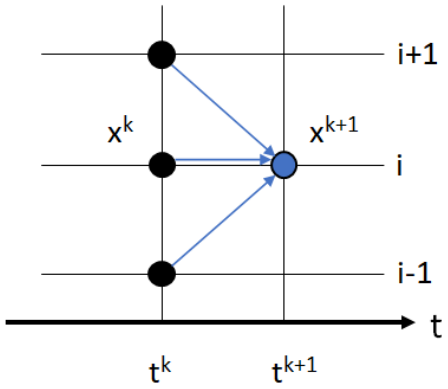

Figure 10: Schematic functionality of the explicit Euler discretization method for time (t) and spatial (i) discretization

As shown, spatial discretization with the symbol *i* is also possible, but not needed for solving the model equations used in this thesis. Stability issues of this discretization method occur, if the time step is too big. The critical time step for biological models,

where whole fermentations with a timespan over several hours are described, is magnitudes larger than the time steps used in the in-house developed CFD tool. The time step size used for simulations in this thesis is 0.000038085 s. Thus, stability issues do not occur and are not further considered in this thesis. As shown in the next section, small time steps are the reason for different challenges occurring in simulations of full bioprocesses.

2.5 Problem outline

In general, the problems in the simulation of full bioprocesses can be attributed to the current state of available computational power. By modelling a fermentation based on the approach described above, different kinds of challenges occur and are outlined in the following chapter.

Pellets

As already mentioned, microorganisms are represented with pellets in this thesis. Due to the computational effort for solving the pellet motions, the real number of microorganisms can currently not be modelled. However, goal is to calculate simulations with enough pellets so that the results are independent of their number. Haringa et al. suggest a comparison between averaged concentrations of Euler-Euler and Euler-Lagrange simulations to validate the approach [15]. An estimation of a certain number of pellets for the Euler-Lagrange approach of Haringa et al. exists but is not applied due to the different approach in pellet modelling, as explained in Section 1.3. Different simulation runs with varying number of pellets are carried out to give more insight into the effect of the number of pellets. The maximum number of pellets is set with 900,000 to maintain fast simulations. It is slightly larger than the number of fluid calculation cells.

Fermentation time

Typical fermentations take several hours to days. Simulating one hour real-time would take about 95 million time steps with a duration of Δt=0.000038085 s. Thus, such simulations would be computationally demanding. In order to accelerate the simulations, the model equations describing the growth of a substance are multiplied with a factor to accelerate the fermentation. Larger time steps for the biological model can be accomplished with this method.

Oxygen transfer

To utilize the tool's own oxygen modelling capabilities, simulations without the OTR of the biological models are carried out. An acceleration of the gas flow rate would lead to a flooding of the reactor and a resulting smaller time step, both unwanted impacts. The first one is not a feasible production state and shorter time steps increases the simulation time. Another way to accelerate the oxygen transfer between air bubbles and the liquid phase is to multiply the oxygen transfer rate with an acceleration factor.

The occurring multiphase regime in the reactor can be described by the dimensionless Flow (Fl) and Froud (Fr) number, as shown in Equation 2-18 and 2-19.

$$Fr = \frac{N^2 * D}{g} = \frac{(190/60)^2 * 0.147}{9.81} = 0.05 \quad (2\text{-}18)$$

$$Fl = \frac{\dot{G}}{N * D^3} = \frac{0.71/3{,}600}{190/60 * 0.147^3} = 0.02 \quad (2\text{-}19)$$

In these dimensionless Equations, *N* represents the stirrer speed, *D* the stirrer diameter, *g* the gravitational acceleration and $\dot{G}$ the gas flow rate. The flow regime based on these numbers is defined as vortex cavities without recirculation [16]. Vortex cavities are low pressure zones behind a stirrer blade. Gas bubble accumulation occurs in these areas and bubbles are not recirculating [4]. This regime remains the same during all simulations. The occurring flow regime can be described by the stirrer Reynolds (Re) number, as shown in Equation 2-20.

$$Re = \frac{\rho * D^2 * N}{\mu} = \frac{1{,}000 * 0.147^2 * (190/60)}{0.001} = 68428.5 \quad (2\text{-}20)$$

The parameter μ represents the dynamic viscosity in Pa·s. The Reynolds number of 68428.5 describes a fully turbulent flow regime in the reactor [16]. For further information of occurring flow regimes and mixing characteristics, "The Handbook of Industrial Mixing" is highly recommended [16].

As shown in Figure 11, the approach with the accelerated oxygen transfer between bubbles and the liquid phase does not lead to satisfying results. Main reason is that the mass transfer of dissolved oxygen between the calculation cells without bubble contact is

to slow for an accelerated fermentation, because it represents real (not accelerated) process behaviour.

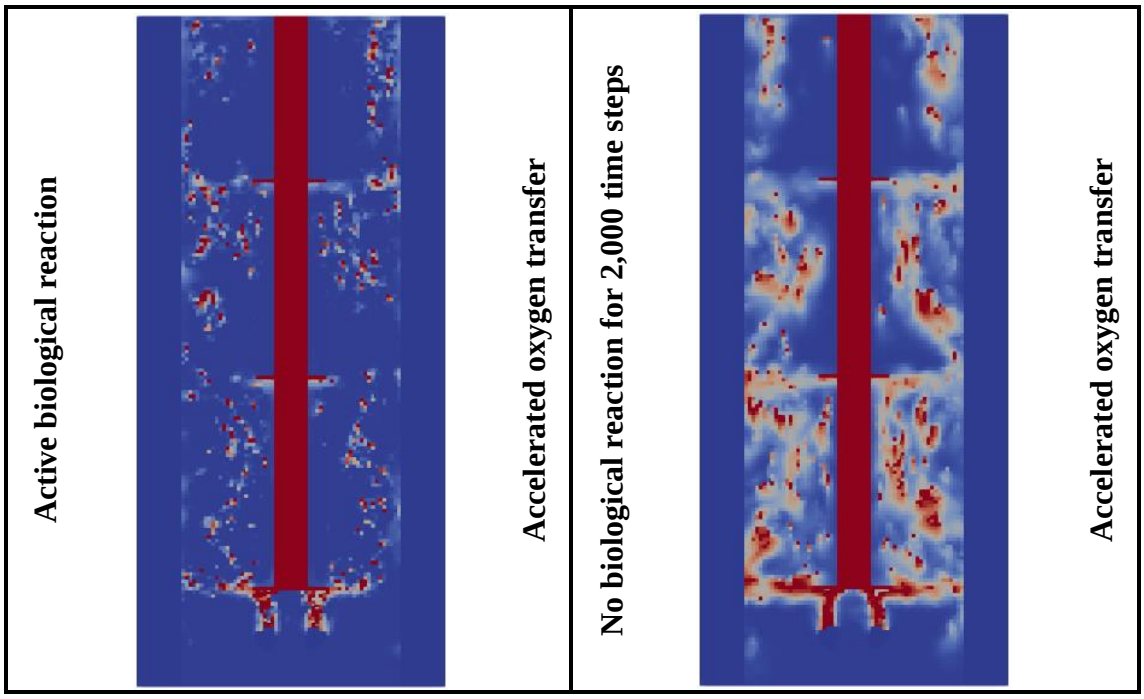

Figure 11: Oxygen concentration with accelerated oxygen transfer between bubbles and the liquid phase with and without an accelerated (3500-fold) biological model. Red calculation cells indicate oxygen saturation of the fluid

The non-accelerated oxygen mass transfer between calculation cells with and without bubble contact leads to a very slow production rate for the accelerated fermentation, because an adequate amount of oxygen is not available in the whole bioreactor. As shown on the left side of Figure 11, the active and accelerated biological model consumes almost the whole available oxygen. Slightly more than 20,000 bubbles are not able to provide the bioreactor with enough oxygen in an adequate time, although the biological model is deactivated, as shown on the right side of Figure 11. Another way to utilize the tool's oxygen model could be the division of the reactor volume in several zones with the size of many calculation cells, where the zones' oxygen concentrations are defined by the number of bubbles in them. However, the biological models' OTR modelling approach is applied, because it can be accelerated. Additional benefit of the applied approach is the lack of modelled bubbles and in further consequence, a faster simulation.

To prove the presented strategies for process acceleration, various verification cases are carried out and presented in the following chapter. An overview about carried out simulations is also shown.

3 Model cases

Typical bioprocesses in stirred reactors are carried out batch- or fed-batch wise. The modelled bioprocesses in this thesis are carried out in batch processes during fermentation. Reaction participants are feed in a fed-batch phase into the reactor. This period is called start-up phase in this thesis. A schematic overview is about the phases is shown in Figure 12.

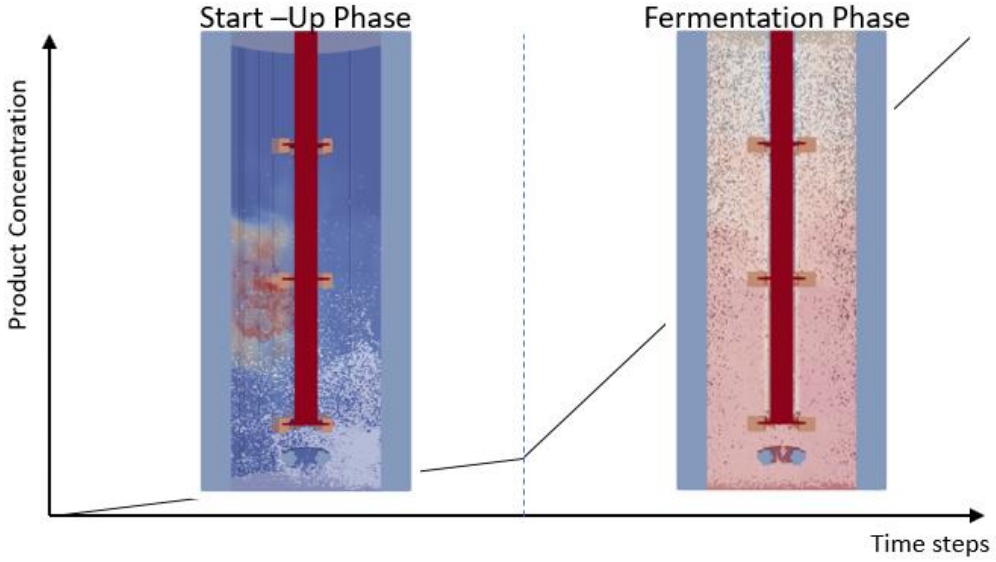

Figure 12: Model approach for a full bioprocess with the in-house developed tool, consisting of a fed-batch start-up phase and a fermentation phase with accelerated biokinetics

The product concentration increases in the fermentation phase due to biokinetics acceleration. An industrial reactor size and geometry is chosen to demonstrate simulation capabilities necessary for the economic analysis. Goal of the start-up phase is preparing the reactor for the following fermentation, which means the feed of reaction participants and pellets as well as mixing the substances. Biomass feed and pellet feed are on the bottom of the reactor while substrate feed is located slightly higher. Exact positions and further reactor geometry parameter are shown in the appendix in Table 10-3.

3.1 Verification cases

As already mentioned, the simulation should not be influenced by the number of pellets. Simulation runs of the gluconic acid's fermentation phase with different number of pellets and time steps (C1, C2 and C3) and a Euler-Euler (E-E) simulation (C4) are carried out. The aim of these simulations is to check the effects of the number of pellets and the deviations to a pellet-free simulation. The gluconic acid fermentation is considered in the verification cases, because a Euler-Euler simulation can be implemented. For the yeast fermentation, a Euler-Euler modeling is not possible due to the necessary intracellular variables. Simulation parameter of the verification cases are shown in Table 3-1.

Table 3-1: Parameter for the verification cases C1, C2, C3 and C4 of the gluconic acid fermentation phase

Parameter	Unit	C1	C2	C3	C4
Time steps	-	1,000,000	450,000	900,000	225,000
Time step duration	s	$3.8 \cdot 10^{-5}$	$3.8 \cdot 10^{-5}$	$3.8 \cdot 10^{-5}$	$3.8 \cdot 10^{-5}$
Reactor Volume	m^3	0.154	0.154	0.154	0.154
Stirrer speed	1/min	190	190	190	190
Number of pellets	-	350,000	900,000	900,000	E-E
Acceleration factor	-	5,675	7,000	3,500	11,350
Modelled real time	h	60	35	35	27

A direct comparison to the Euler-Euler simulation enables a rating of the Lagrangian point of view. Parameters for the start-up phase of the gluconic acid bioprocess can be found in the next section. The modelled real time can be calculated by multiplying the acceleration factor with the number of time steps and the time step duration. Computation times of C1, C2, C3 and C4 are 27,5 h, 14 h, 28 h and 7 h, respectively.

3.2 Verification

The reference for all verification cases is the biomass concentration trend, calculated with the simple biological model presented in Chapter 2.2. The biomass concentration trend is chosen because it is the determining parameter of the gluconic acid biological model. Concentration profiles of the verification cases as well as the reference are shown in Figure 13.

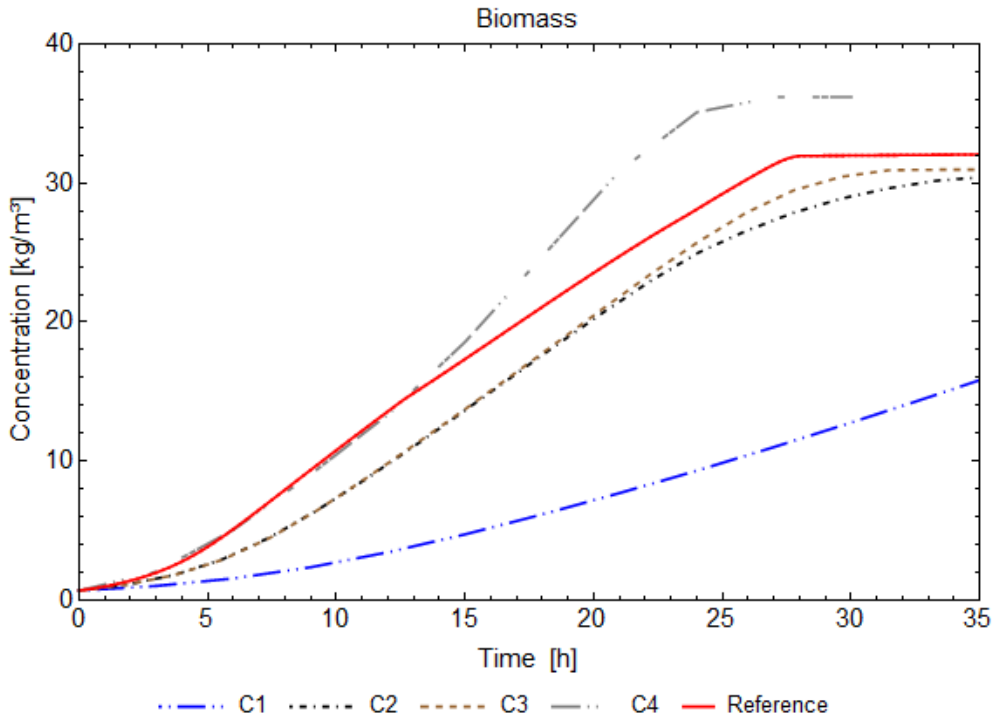

Figure 13: Comparison of the verification cases to the reference provided by the simple biological model. C1: 350,000 pellets; C2: 900,000 pellets and 450,000 time steps; C3: 900,000 pellets and 900,000 time steps; C4: Euler-Euler approach

The Euler-Euler simulation (C4) and the Euler-Lagrange simulations (C2, C3) with 900,000 pellets presents the most accurate results compared to the reference. The mean absolute percentage error (MAPE) of C2 is with 4.0% larger than for C4 with 1.9%. Doubling the time steps of C2 leads to a decrease of the MAPE to 3.8%, as shown with the simulation case C3. The equation for the mean absolute percentage error can be found in Chapter 10.2, Equation 10-5. C4 results in a higher biomass concentration with a faster growth in comparison to the reference. It is assumed that the reason for the deviation after 15 h is the underlying approach of the Euler-Euler simulation, namely that fermentation

takes place in the whole bioreactor. The reference model is based on material balances and parameters derived from real experiments, where fermentation takes not place in the whole reactor due to dead volumes without bacteria. However, the Euler-Euler simulation is still very close to the reference.

The Euler-Lagrange simulations C2 and C3 show more accuracy to the reference model regarding reaction rate and final biomass concentration during the whole process although the Euler-Euler simulation C4 has a smaller MAPE and models the first 15 h of the fermentation without a significant error. The main reason of the slower fermentation of C2 and C3 is that the pellets cause larger concentration gradients in the reactor than the Euler-Euler simulation, as shown later in Chapter 4.1. Additionally, not every calculation cell is occupied with a pellet. Especially the space behind the baffles is more affected as in the Euler-Euler simulation, as shown in Figure 14. These areas are less likely to be in contact with pellets due to the rotating flow field in the stirred bioreactor. Thus, the dead volumes cause a slower fermentation, because the biological reaction does not occur in the whole bioreactor.

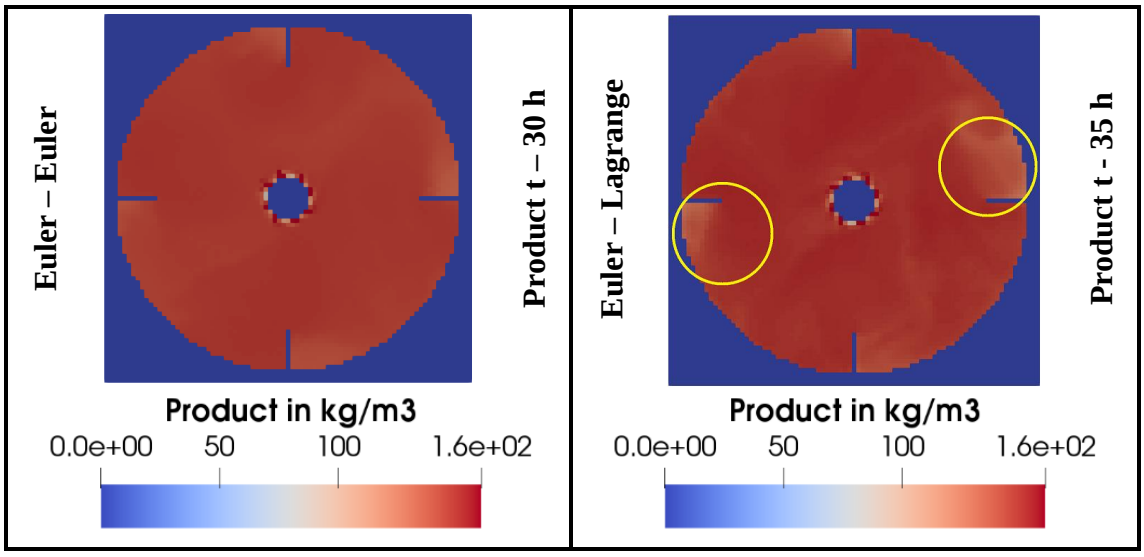

Figure 14: Comparison of the top view of a Euler-Euler modelled (C4) and a Euler-Lagrange (C2) modelled bioprocess after the last time step. The yellow circles mark the areas behind baffles with lower concentrations than in the Euler-Euler simulation

An issue needs to be addressed is the viscosity. It rises in real fermentations due to the growth of biomass [11]. According to the publication, a tenfold increase of the viscosity leads to a 25% decrease of production rate for this fermentation due to a decreasing K_La value. This effect is considered in the simulations with an averaged K_La value, also provided by the biological model [10]. If the tool's oxygen modelling approach would be applied, the increase of the viscosity due to biomass growth needs to be considered with correlations, because the K_La value is not constant [11]. However, due to the applied oxygen modelling approach of the biological model, this consideration is not necessary, and viscosity of water can be used.

The result of C1 is not satisfying due to the large deviation to the reference. By comparing simulation results of C1 with C2 and C3, it is obvious that the number of pellets has a major impact on the accuracy of the results. Figure 15 shows the pellet cell density for the cases. Red dots represent calculation cells which contains more than five pellets.

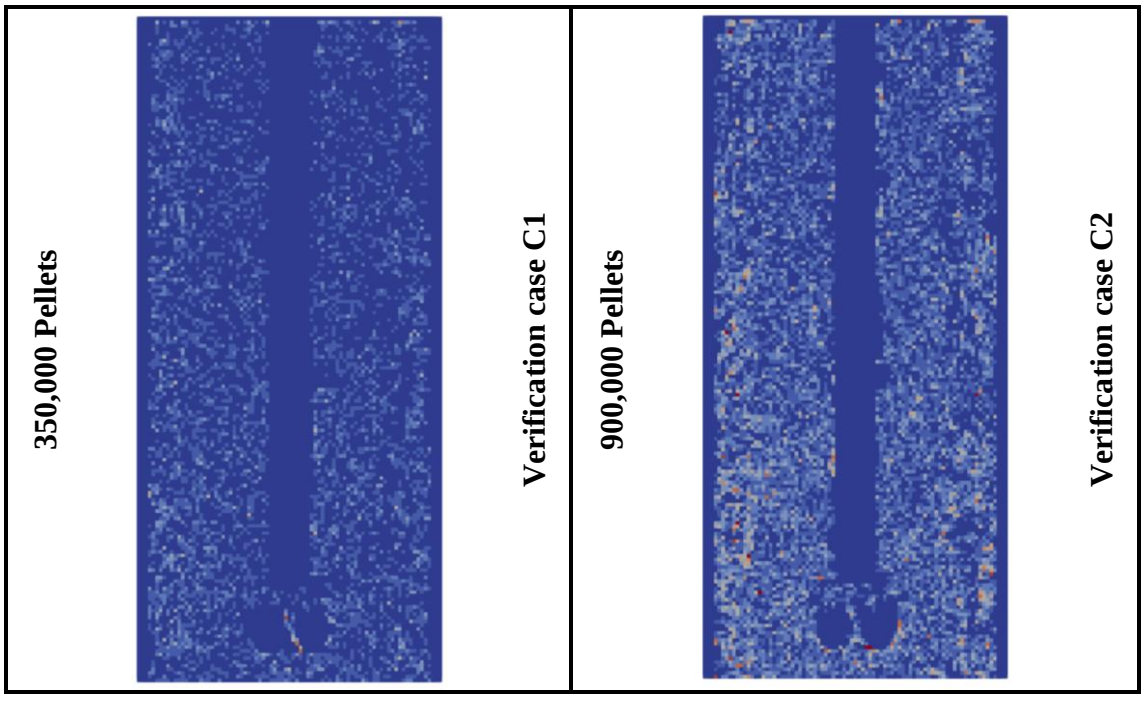

Figure 15: Pellet cell density with 350,000 (C1) and 900,000 (C2) pellets in contrast to 850,000 calculation cells of the modelled bioreactor

Setting the number of pellets higher than the number of fluid calculation cells is highly recommended if the Euler-Lagrange approach is used. Using 350,000 pellets with 850,000 calculation cells results in too much pellet-free calculation cells so that the

fermentation process is to slow. Slightly more pellets are in the lower half than in the upper half of the bioreactor.

Doubling the time steps leads to a decrease of the MAPE, as shown with the simulation case C3. Reason for this phenomenon is that the real simulation time is twice as long as for C2. This means that the stirrer has twice as much revolutions and the fluid field has twice as much time to balance spatial concentration gradients, especially at the end of the fermentation where biomass production rate decreases. However, final biomass concentrations are almost the same for C2 and C3.

To sum it up, the Euler-Euler simulation C4 and the Euler-Lagrange simulations C2 and C3 show high accuracy compared to the reference. The Euler-Lagrange approach is used to model the two bioprocesses, because it enables the consideration of intracellular variables, the tracking of a pellet's lifetime and it shows higher accuracy regarding average reaction rate and final concentrations. Applied parameters are presented in the next section. Besides the major impact of the pellet number, the number of time steps also slightly influences the accuracy of the Euler-Lagrange approach. However, modelled bioprocesses in Chapter 4 are simulated with less than 500,000 time steps due to faster computations. The simulation parameters for the modelled bioprocesses are shown in the next section.

3.3 Model cases: Bioprocesses

Since the overall goal of the simulation part is the modelling of bioprocesses with new biological models to demonstrate the tool's capabilities, the already presented gluconic acid fermentation and yeast fermentation are modelled. Parameter setting for each bioprocess for the starting phase is shown in Table 3-2. In both fermentations, the 154 L reactor is inoculated with 0.1 kg biomass. The gluconic acid start-up phase is modelled for 60 s, the yeast start-up phase for 45 s real-time.

Table 3-2: Parameter for the start-up phase for the yeast and gluconic acid fermentation

Parameter	Unit	Gluconic Acid	Yeast
Time steps	-	1,600,000	1,150,000
Time step duration	s	$3.8 \cdot 10^{-5}$	$3.8 \cdot 10^{-5}$
Reactor Volume	m^3	0.154	0.154
Stirrer speed	1/min	190	190
Substrate feed amount	kg	25	2
Biomass feed amount	kg	0.1	0.1
Number of pellets	-	900,000	900,000
Fluid calculation cells	-	~850,000	~850,000

Parameter setting for the fermentation phase for each simulation is shown in Table 3-3. In contrast to the start-up phase, a time step acceleration factor for tuning the biokinetics is considered.

Table 3-3: Parameter for the fermentation phase for the yeast and gluconic acid fermentation

Parameter	Unit	Gluconic Acid	Yeast
Time steps	-	475,000	300,000
Time step duration Δt	s	$3.8 \cdot 10^{-5}$	$3.8 \cdot 10^{-5}$
Reactor Volume	m^3	0.154	0.154
Stirrer speed	1/min	190	190
Substrate feed	kg	0	0
Biomass feed	kg	0	0
Number of pellets	-	900,000	900,000
Acceleration factor	-	7,000	5,000
Real time	h	35	16

The time step acceleration factors are derived from the fermentation time shown in Section 2.2 and 2.3 and a feasible number of time steps for a fast simulation run-time. The gluconic acid fermentation is the same as in the verification case C2. Computing the yeast fermentation takes about 10 h.

All simulation parameters that are not provided in this section are shown in the appendix in Section 10.3. Results, analysis and discussion of the fermentations are shown in the next section.

4 Analysis of the modelled bioprocesses

The previously described start-up phase and fermentation phase of the bioprocesses are reviewed in the following analysis. The lifeline of one pellet is discussed for the start-up as well the fermentation phase. A comparison of the modelled bioprocesses with the simple biological models is also carried out.

4.1 Gluconic acid fermentation

Start-Up phase

The start-up phase takes about 60 s of real-time. In this period and no parameters are accelerated. Thus, a significant gluconic acid production cannot be expected, because the whole fermentation takes several hours. However, a recorded pellet lifetime in the start-up simulation gives more insight into the distribution of reaction participants in the reactor and is therefore analyzed. Figure 16 shows one pellet's changes over time of the recorded production rate. The mean of 2,000 pellets' production rates is also shown.

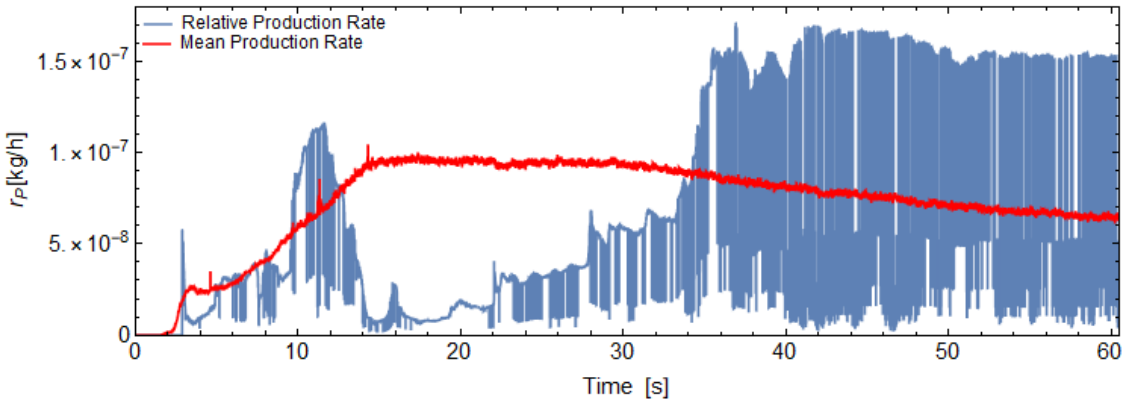

Figure 16: Observed relative production rate of one pellet (blue) and mean production rate of 2,000 pellets (red) during the start-up phase

As shown, the production rate in the start-up phase has its maximum after 35 s. At the beginning of the fermentation, no oxygen, substrate, pellets and biomass are available. Thus, the fermentation starts after several seconds when a minimum level of added substances is reached. The high frequency of vertical outliers is caused by more than one pellet sharing the calculation cell. Therefore, less substrate, oxygen and biomass are available for one single pellet. In comparison to one pellet, the mean production rate does

not fluctuate and reaches $6 \cdot 10^{-8}$ kg/h. After 30s, the mean production rate decreases because the substrate and biomass supply stop but more and more pellets enter the bioreactor and share the available reaction participants.

The peak in the production growth rate of the observed pellet at 10 s is caused by passing the substrate feed, as shown in Figure 17.

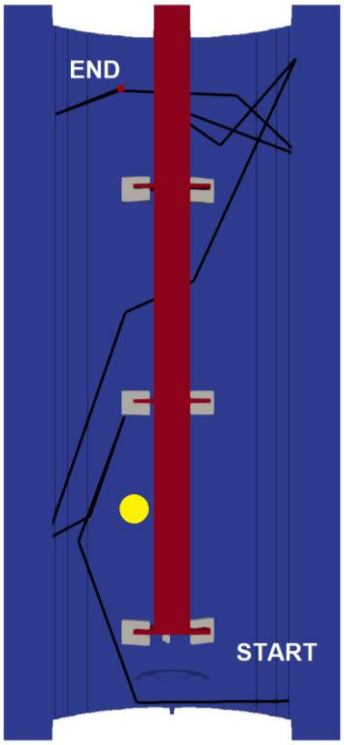

Figure 17: Pathway of a pellet during the first 30 seconds of the start-up phase. The substrate feed is represented by the yellow dot

The considered pellet passes the substrate feed position after several seconds. In this section, substrate concentration during the start-up phase is higher than in the upper part of the reactor, as shown by the slower production rate after leaving the marked location. After 35 s, feed of all substances is completed and the production rate of the considered pellet increases to its maximum.

Fermentation phase

In the fermentation phase, the biological reaction is accelerated to simulate the full fermentation in a feasible time. Pellets and stirrer experience no acceleration and proceed their behaviour of the start-up phase. Figure 18 shows the production rate in the fermentation phase of the particle considered in the start-up phase.

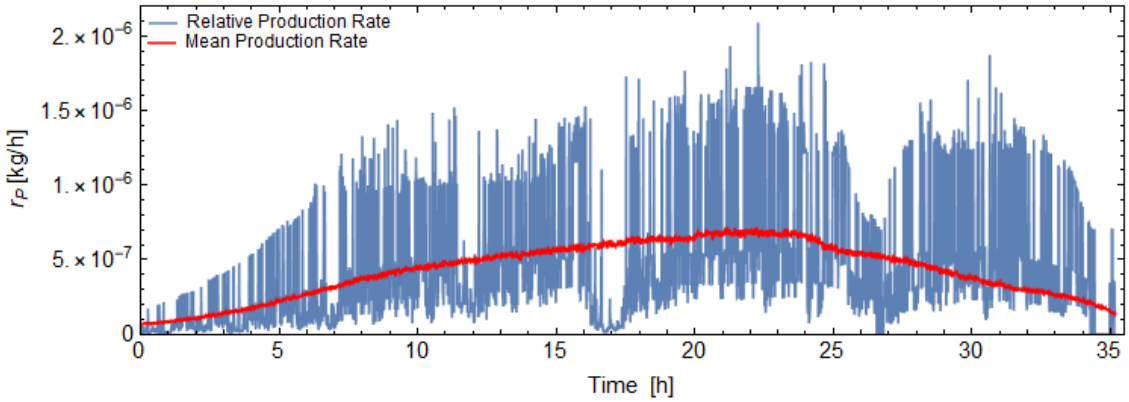

Figure 18: Observed production rate of one pellet (blue) and mean production rate of 1,000 pellets (red) during the accelerated fermentation phase of the gluconic acid fermentation

In comparison to the recorded production rate of the start-up phase shown in Figure 16, the maximum value of the mean production rate of 1,000 pellets is $7 \cdot 10^{-7}$ kg/h in the fermentation phase. Outliers in the production rate of one pellet occur in the same way as in the start-up phase. Interesting is the increase and decrease of the production rate of the pellets. Reason for the increase in production is the growing biomass for this pellet, because it is linked directly to the production rate. After about 25 h, substrate in lower parts of the reactor is almost depleted and the specific growth rate μ decreases.

Figure 19 shows concentration profiles for substrate, oxygen, biomass and product after 27 h of fermentation. Substrate in the lower half of the reactor is completely depleted due to a higher density of pellets in this area, as shown in Figure 15. This results also in a higher product concentration in this reactor area. Same as in the original model, the fermentation in a cell is stopped after the depletion of the substrate [10].

Figure 20 shows the overall concentration trends of the considered fermentation compared to the reference trends of the simple biological model. Oxygen concentration during the fermentation is almost zero. Therefore, oxygen concentration in the upper reactor half after 27 h is almost zero, because substrate is still processed to gluconic acid. This phenomenon is shown in Figure 19. In the lower half, substrate is completely depleted and oxygen level increases. The dots with higher oxygen concentration in the upper half are free from pellets. Thus, the biological model is not active in these areas.

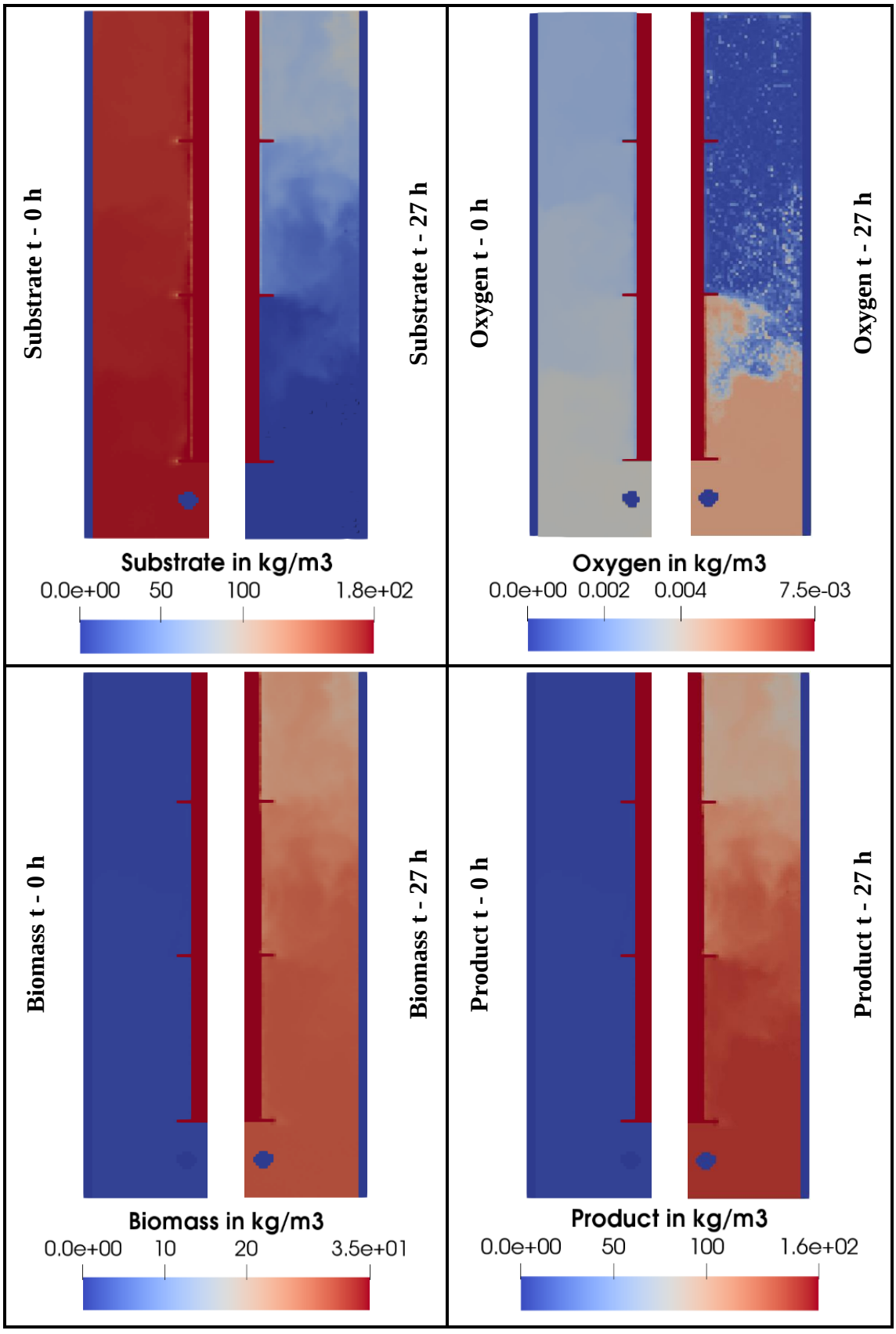

Figure 19: Concentration profiles of gluconic acid fermentation's reaction participants

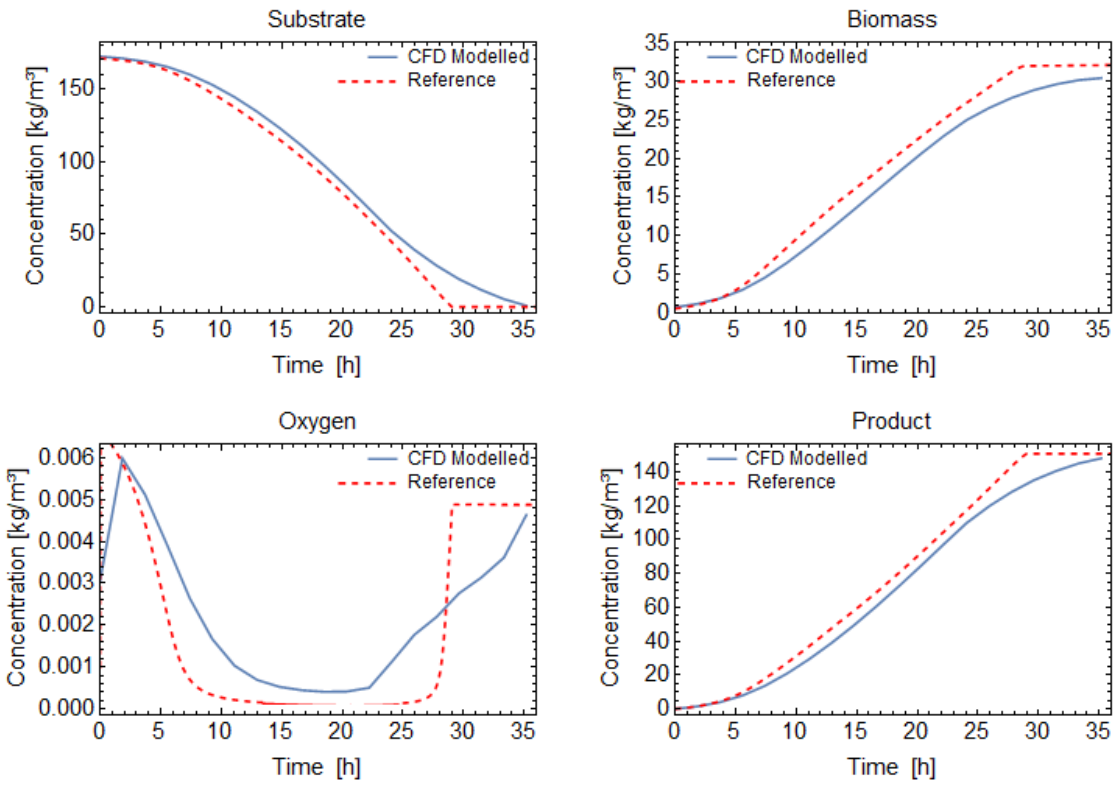

Figure 20: CFD modelled and averaged concentration trends of the gluconic acid fermentation in comparison to the results from the simple biological model

By comparing the modelled trends with the reference trends, the accurate representation of the reference is obvious. Spatial concentration gradients are the reason for the significant derivations of the trends after 30 h. As shown in Figure 20, the lower and upper bioreactor parts have different concentrations. Thus, the fermentation is finished in the lower half of the reactor much earlier than in the upper half and overall fermentation velocity decreases in the last hours. In comparison to Figure 8, the lag phase is skipped due to higher initial biomass concentration.

In the next section, the already presented yeast fermentation with consideration of intracellular variables is analysed and discussed.

4.2 Yeast fermentation

Hence the start-up phase for this process is similar to the previous one, only the fermentation phase is analyzed. Intracellular variables are the three enzyme concentrations for each metabolic pathway and the carbohydrate storage.

Fermentation phase

Different to the gluconic acid fermentation, the yeast microorganisms process their produced ethanol if no substrate is available. Growth rate of one pellet and the mean growth rate of 1,000 pellets is presented in Figure 20.

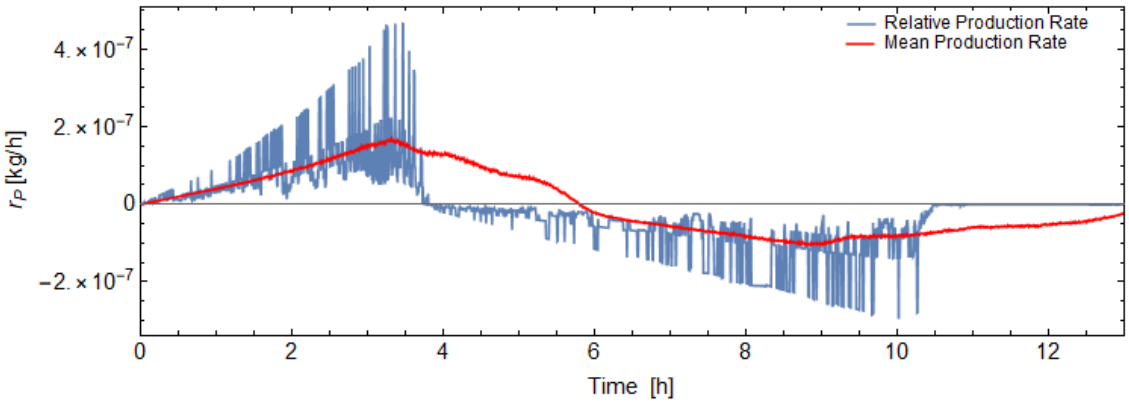

Figure 21: Observed production rate of one pellet (blue) and mean production rate of 1,000 pellets (red) during the accelerated fermentation phase of the yeast fermentation

The growth rate switches from positive to negative due to a depletion of the available substrate. The growth rate trend for one pellet differs to the mean growth rate trend significantly, because the considered pellet moves through calculation cells without glucose nor ethanol. Thus, production stops much earlier for the pellet than the average pellet.

Figure 22 shows concentration profiles of the fermentation's start and after eight hours. There is also a significant spatial concentration distribution recognizable. Substrate is not homogenously distributed at t=0 due to a shorter start-up phase. After eight hours, the substrate is completely depleted and the microorganisms switched to the ethanol oxidation metabolism to process the available ethanol.

In Figure 22, the concentration profiles of substrate, biomass, product and oxygen for the modelled yeast fermentation are presented. The trends are compared with reference trends provided by the simple biological model.

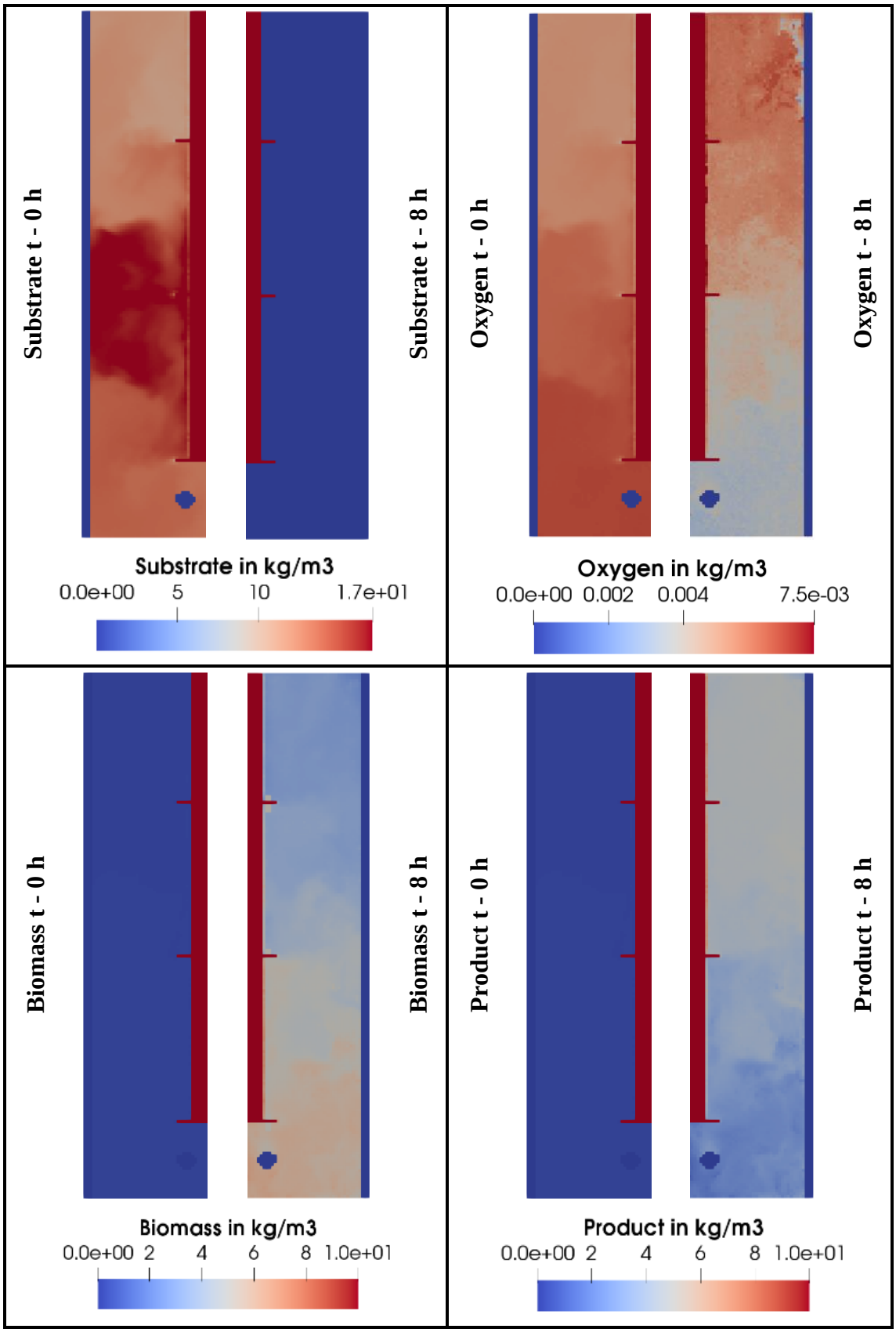

Figure 22: Concentration profiles of yeast fermentation's reaction participants

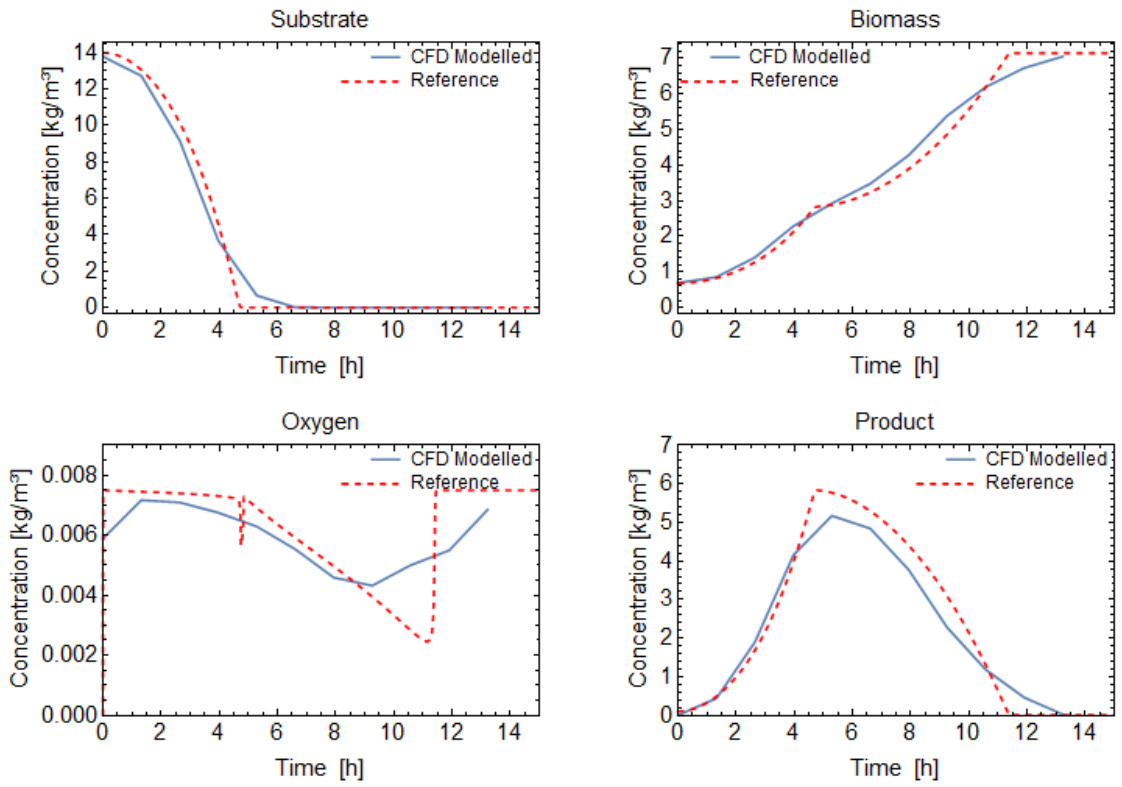

Figure 23: CFD modelled and averaged concentration trends of the yeast fermentation in comparison to the results from the simple biological model

In general, the simulation can represent the simple biological model very well. The biomass trend shows a mean absolute percentage error of 0.5% with respect to the biological model. During the process, 1456 kJ heat and 1.647 kg CO2 are produced. These values differ to the ones presented in Section 2.3 due to different initial concentrations and a smaller reactor volume. Same as in the previous fermentation, the Euler-Lagrange approach predicts slightly more time necessary for a complete fermentation in comparison with the biological model.

To sum it up, the two simulated bioprocesses accurately represent the references, provided by the simple biological models. The implemented extensions increased the tool's biological capabilities and proved, that full bioprocesses can be simulated in an adequate timeframe. Additionally, the implemented framework to simulate biological models with a Euler-Lagrange approach increases the tool's biological capabilities. In the following chapters, the marketability of the tool is rated and a development of a business

model is carried out. The presented capabilities during the carried-out simulations build the framework for the following rating of strengths and weaknesses of the tool.

5 Market and competitive analysis

Essential part of rating the tool's marketability is the market analysis. It enables insight into the current and future market development for its fields of application. Before the market analysis can be executed, the target market needs to be defined. Based on the defined target market, the market analysis is carried out with focus on the future development of the biopharmaceutical industry. Future trends that may be an opportunity or a threat for the market are also considered. A further market analysis based on a later derived business model is carried out in Section 7.3.
To rate the competitiveness of the tool, a comparison with several commercial CFD tools is done. Special focus of the market and competitive analysis is on industrial-scale bioreactors.

5.1 Target market

The in-house developed CFD tool differs to competitive CFD tools mainly in the field of application and in further consequence, in the target market. While most commercial CFD tools are designed to cover a wide range of applications, the in-house developed CFD tool is focused on stirred and aerated bioreactors. Thus, potential users are companies in traditional fields of application and engineering of bioreactors like the pharmaceutical industry, the food industry, the chemical industry and the agricultural industry [17]. Bioreactor manufacturer are also potential users because of their need of optimization and interest in deeper process understanding. Table 5-1 shows typical applications for previous listed industries, exemplary companies with classical processes and corresponding reactor capacities.

Table 5-1: Typical applications of industrial-scale bioreactors in different industries with exemplary manufacturers

Industry	Manufacturer	Product	Volume	Reference
Pharmaceutical	*Lonza Biologics*	Therapeutic proteins from mammalian cells	20 m³	[18]
Pharmaceutical	*Roche, Johnson & Johnson*	Therapeutic proteins from bacteria	250 m³	[19]
Food	*BASF, Novozymes*	Yeast	30-80m³	[19]
Agricultural	*Evonik, ADM*	L-Lysine	500m³	[20]
Chemical	*ADM, Poet*	Bioethanol	70m³	[21]

As shown, large-scale bioreactors are widely used in the biotechnological industry. In 2008, 70% of all bioreactors were non-mixed and non-aerated systems, 20% mixed and aerated systems and 10% non-mixed and aerated systems [22]. Besides stirred bioreactors, wave-mixed and shaken bioreactors are also used in industry [23].
The in-house developed CFD tool is originally conceptualized for a pharmaceutical company. Therefore, it is customized to this field of application. Due to the specialization in this field of application and thus the gathered experience, the pharmaceutical sector is considered as the most important industry of the target market.

In general, API's of novel medicines are produced with chemical synthesis, fermentation or with extraction of vegetable or animal tissues [24]. Figure 24 shows the distribution of the chemically or biologically produced APIs of all *FDA* drug approvals over the last 20 years.

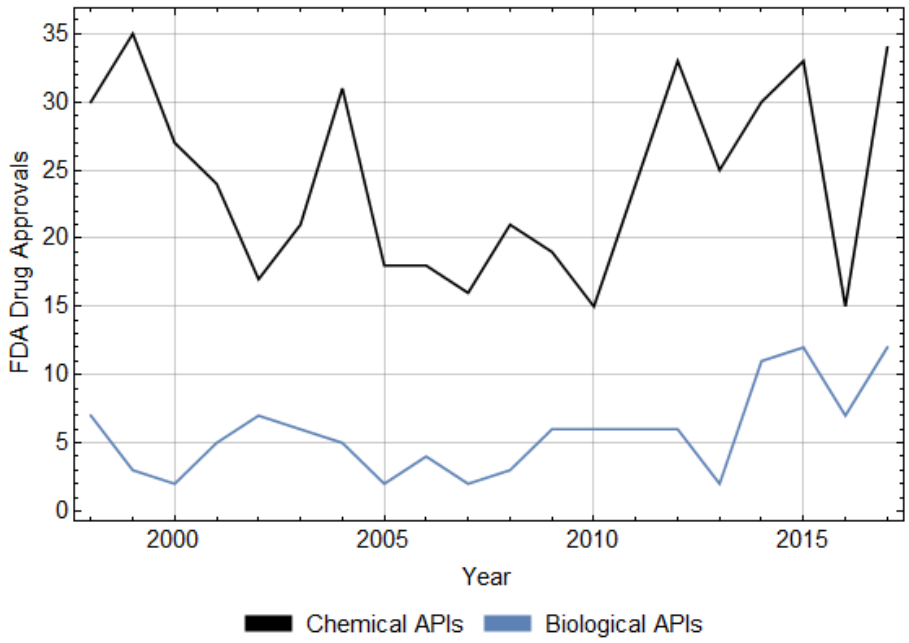

Figure 24: Novel Chemical and biological entities approved by the *FDA* between 1998 and 2017 [25]

As shown, drugs based on extraction of vegetable or animal tissues are no subject to approval due to their weak effects. However, an increase of drug approvals with biologic molecules compared to total approvals since 2013 is obvious. This trend shows the growing importance of biopharmaceutical drugs. Thus, the following section gives further insight into the biotechnological market with focus on the biopharmaceutical sector.

5.2 Market analysis

After the determination of the target market, the market analysis with focus on the biopharmaceutical sector can be carried out. Special attention is on the change of bioreactor capacity in the next few years. A general forecast about the CFD market's future development enables an estimation of future acceptance of CFD tools and is carried out in the following.

The usage of CFD tools is predicted to rise significantly over the next years. A report estimates that the revenue generated with selling CFD tools will reach 2180 million US $ in 2022 [26]. 2016, the global CFD software revenue was at 1,277 million US $.

According to the numbers, the report predicts a compound annual growth rate of 9.32% which results in nearly doubling the revenue of 2016 in 2022. Unfortunately, no representative numbers about the development of CFD usage in the pharmaceutical industry can be found. However, a growth of the CFD usage in biopharmaceutical industry can also be expected due to the large growth of the general CFD market and the benefits of modelling bioreactors with the CFD approach, already presented in Chapter 1. A look on the current situation of the biotechnology market reveals the importance of this industry. 2,259 biotechnology companies were counted 2015 in Europe [27]. Worldwide, all pharmaceutical companies generated a revenue of 1,105.2 billion US $ [28]. The growth of the worldwide pharmaceutical sector is predicted with 160% until 2030 [29]. The change of the bioreactor and fermenter market is also highly significant. In 2016, the global bioreactor and fermenter industry generated a revenue of 1,779.1 million US $ and will reach 4,461 million US $ in 2024 with a compound annual growth rate of 12.2% [30]. However, in the last fifteen years the willingness to expand biopharmaceutical facilities changed significantly. According to a survey in the biopharmaceutical industry, 79% of survey participants in 2003 planned a facility expansion in five years. On the other side, less than 30% of the participants in 2018 report planning facility expansions in five years [31].

It is assumed that the application of CFD tools correlates directly with the change of bioreactor capacity due to scale-up simulations, optimizations or other engineering tasks. An accurate determination of the global bioreactor capacity is very difficult. It is estimated, that the current global fermentation volume for red biotechnology (mainly large therapeutic proteins and monoclonal antibodies for the pharmaceutical market) is about 5,000 m^3 and the volume for white biotechnology (different products for different markets and applications) is 350,000 m^3 [19].
Another report of 2016 provides detailed insight into the future and present bioreactor capacities for mammalian cell culture fermentation [32]. Mammalian cell fermentation can be assigned to red biotechnology. The report reveals that there are about 900 biopharmaceutical products in some stage of clinical development in the United States and Europe. 77% of these products are produced in mammalian cell culture systems. The remaining 23% are mainly produced with microbial metabolites. Figure 25 shows the

volumetric capacity needed to support the clinical development and commercial sales of all pipeline mammalian cell culture-based products.

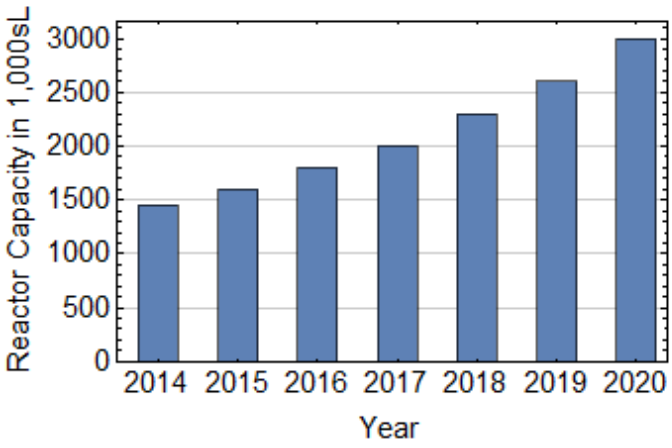

Figure 25: Forecast of volumetric capacity needed to meet mammalian cell culture manufacturing demand [32]

As shown, the required capacity is expected to rise significantly. The report provides also insight into the development of installed capacity, as shown in Figure 26.

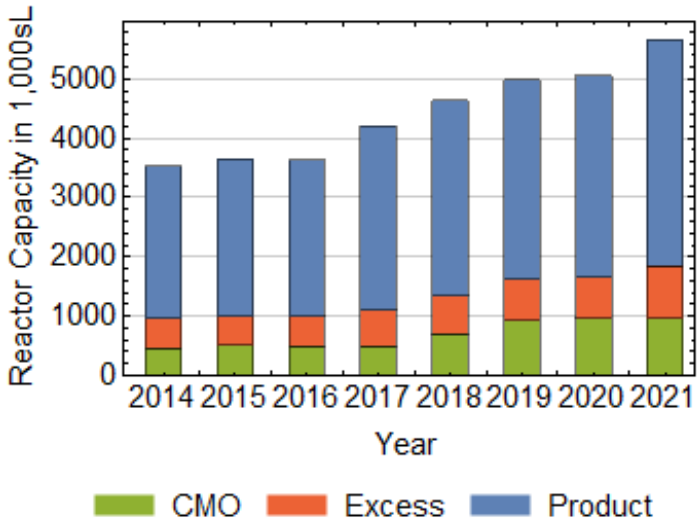

Figure 26: Current mammalian cell culture manufacturing capacity of CMOs, excess companies and product companies [32]

In 2021, the mammalian cell culture supply is predicted to grow to approximately 5,600 m³. The main part of installed production capacity is provided by product companies. Excess companies (companies that sell or make available any excess manufacturing

capacity) and CMOs (Contract Manufacturing Organization) are predicted to increase their capacity faster than product companies.

A comparison between Figure 25 and Figure 26 shows a significantly excessive supply of mammalian production capacity in respect to the corresponding demand. Many manufacturers consider full utilization at 70-80% to have reserves for product changes, preventive maintenance and facility upgrades. The capacity demand is predicted to grow at a significantly larger rate as the installed one. The report summarizes also capacity extension projects of the top 10 capacity holders of the pharmaceutical industry. Almost all considered companies plan to extend their bioreactor capacities.

According to Table 5-2, 50% of products which are in Phase 2 and Phase 3 of clinical development today can be produced with 5,000 L reactors or smaller once they are ready for market. The remaining 50 % of future products will need bioreactor capacity of at least 10,000 L.

Table 5-2: Bioreactor size distribution of mammalian products in clinical development phase 2 and phase 3 in 2017 [32]

Number of products	**<2,000 L**	**5,000 L**	**10,000 L**	**>10,000 L**
285	118 (41%)	25 (9%)	32 (11%)	110 (39%)

However, a more recent report of April 2017 presents a survey among 199 biopharmaceutical manufacturers and contract manufacturing organizations (CMOs) [33]. The survey focuses on the whole range of current biopharmaceutical products (Mammalian cell culture, microbial fermentation, yeast, insect cells and plant cells).

According to the study, 20.1% of the largest stainless-steel bioreactors in use are equal or larger than 10,000 L. They found an average stainless bioreactor size of 4,718 L. Figure 27 shows the result of the survey. As shown, this study considers laboratory bioreactors as well as industrial-scale bioreactors.

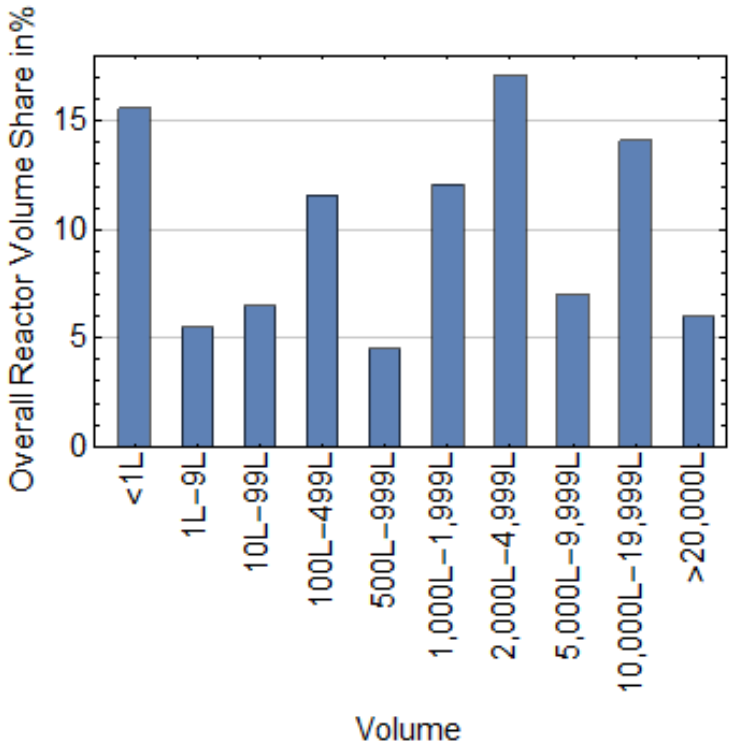

Figure 27: Survey result among 199 biopharmaceutical manufacturers of the stainless-steel bioreactor sizes in use [33]

Based on the presented average stainless bioreactor size and the predicted growth of mammalian cell production capacity, the number of future build bioreactors can be estimated. According to Figure 26, current mammalian cell production capacity is about 4,600,000 L and expected to rise to 5,500,000 L in 2021. By dividing the predicted capacity growth 900,000 L with the average reactor size of 4,718 L, it can be stated that 190 new mammalian cell bioreactors are expected to be built until the end of 2021.

To sum it up, the biopharmaceutical industry is predicted to continue to have strong growth. The total demand for mammalian manufacturing capacity and the capacity itself will also increase in the next few years. 77% of future biopharmaceutical products are based on mammalian cell fermentation. It is expected that the capacity demand will slightly rise faster than the available manufacturing capacity. Based on this forecast and the expected growth of CFD software revenue as well as the benefits of modelling bioreactors with CFD, an increasing demand for CFD tools in the pharmaceutical industry can be predicted. The fact that 50% of new products are going to be produced in

bioreactors with 10,000 L or greater enhance the assumption. However, significantly less willingness to extend biopharmaceutical production capacities in the next five years exist, compared with numbers from 2003. Future trends, which may have a heavy impact on the biopharmaceutical industry are discussed in the following section.

Trends in pharmaceutical engineering

Awareness of trends is a necessary part of every market analysis, because they can either be a risk or an opportunity for a possible market entry. For example, continuous processes are a novel trend in pharmaceutical industry. Like in food production and other industries, main advantage is the acceleration of the process. However, industrial continuous manufacturing for bioprocesses is expected to be applied only in the downstream part of the process, at least for the next ten years [34]. Current advances in continuous fermentation are based on the classic geometry of a stirred tank reactor (perfusion operation) [35]. An industrial establishment of continuous fermentation can therefore be an opportunity for the in-house developed CFD tool, because it relies on the principle of a fed-batch bioreactor. Other future developments which shortens the fermentation time present also an opportunity for the in-house developed CFD tool, because they can be implemented and considered in the flexible tool (i.e. biocatalysts).

Another advance is the rapid installation of new manufacturing facilities and rapid switching between production campaigns, possible with disposable or single-use technologies. Nearly all unit operations are now available as disposable equipment, including disposable bioreactors [34]. There are several economic and ecological benefits by using single-use systems for the pharmaceutical industry. Today, disposable bioreactors are available up to 5,000 L. In 2013, disposables were predicted to reach 20% market share of the bioprocess technologies market until end of 2018 [36]. Due to the significance of the single-use trend, a consideration in the capabilities of a CFD tool for bioreactors is a must. Being able of modelling single-use bioreactors could be an opportunity to take up a leading position on the bioreactor CFD market. For example, non-vertical stirrers are often used in single-use bioreactors and can already be considered by the in-house developed CFD tool.

A survey of 2017 with 199 participants reveals an average single-use bioreactor size of 604 L [33]. This result is based on the largest single-use bioreactor capacities used in a facility. The size distribution is shown in Figure 28. By comparing these results to the size distribution of stainless-steel bioreactors, it is obvious that the trend of single-use bioreactors is currently restricted on small reactors.

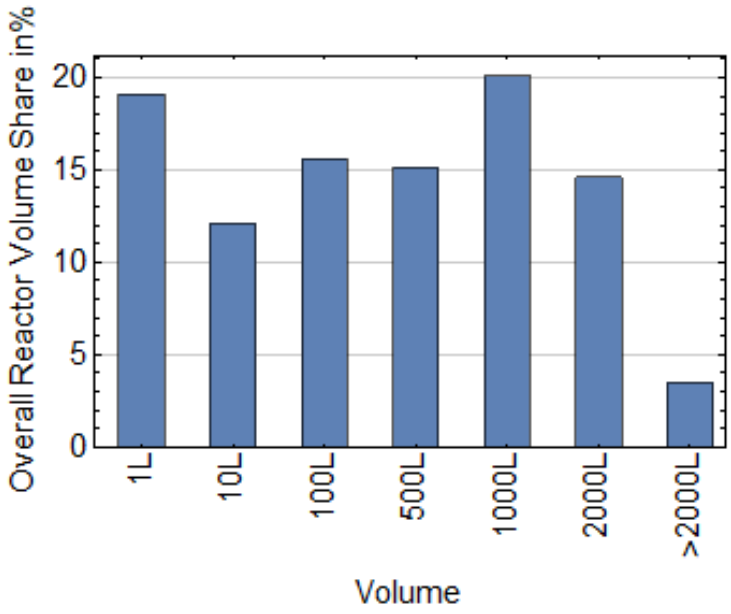

Figure 28: Survey result among 199 biopharmaceutical manufacturers of the single-use bioreactor sizes in use [33]

A novel trend in the pharmaceutical industry is looking at ways to reduce the industry's plant-floor footprint. The company *Amgen* plans to build a new manufacturing facility in Rhode Island with the same output as the company's plant in Singapore, but with a fifth of its size. The downsizing is accomplished with process intensification, which increased the expression rates in bioreactors significantly. 2,000 L reactors can now accomplish the same product amount as ten-year-old 15,000 L reactors [37]. Due to this possible downsizing, this trend is a clear threat for a CFD tool that is specialised on modelling industrial-scale bioreactors.

In conclusion, some novel trends in the pharmaceutical industry could be a chance for the in-house developed CFD tool. Especially the capability of modelling disposable bioreactors carries a huge potential because of their expected increase of use in the pharmaceutical industry. Thus, the single-use bioreactor sector needs special

consideration in a modern bioprocess simulation tool. A clear threat for a CFD tool specialised on modelling industrial-scale bioreactors is the trend of downsizing manufacturing facilities with the help of process intensification. After the derivation of a business model, a further and more detailed market analysis can be accomplished.

5.3 Competitive analysis

This section provides a comparison between capabilities of the in-house developed tool and available CFD tools used for bioreactor modelling. The considered CFD tools are named as competitors in this analysis. Listing of the competitor's capabilities is an important aspect of the competitor analysis, because it enables an identification of strengths and weaknesses of the in-house developed tool. The competitive analysis procedure is based on methods presented in the book "Wettbewerbsanalyse für Ingenieure" [38]. Capabilities of the in-house developed CFD tool are discussed in Chapter 1. The first step of a competitive analysis is the identification of competitors. Table 5-3 shows a selection of the most important ones. The identification is based on the frequency and number of recent publications with focus on bioreactor modelling.

Table 5-3: Direct and indirect competitors

	Direct Competitor		*Indirect Competitor*
Developer	**CFD Commercial**	**CFD Open Source**	**Process Modelling**
Ansys Fluent	■		
Comsol Multiphysics	■		
OpenFoam		■	
Aspen One			■
M-Star CFD	■		

The following discussion points out the capabilities of the previous defined competitors based on scientific publications. Analysed competitors are *Ansys Fluent*, *Comsol Multiphysics*, *OpenFoam*, *Aspen One* and *M-Star CFD*. Focus is a capability comparison

of the in-house developed tool and the defined competitors. Additionally, a general overview about the several competitors is made.

Ansys Fluent

Ansys Fluent is the market leader in the CFD Simulation industry. The stock corporation has more than 3,000 employees and crossed 1 billion US $ revenue in 2017. The majority of publications about CFD modelled bioreactors are based on this tool [39]. Table 5-4 gives an overview about relevant publications made on bioreactor models with *Ansys Fluent*.

Table 5-4: Relevant CFD models of bioreactors based on *Ansys Fluent*

Turbulence	Multiphase Model	Biologics	Reactor Type	Year	Reference
k-ε	Euler-Lagrange	No	Stirred tank (2.3 L & 80 L)	2016	[26]
k-ε	Euler-Euler	Monod-Model & PBM	Stirred tank (70 L & 70 m³)	2014	[27]
-	Euler-Euler	No	CSTR (17 L & 140 m³)	2010	[28]
RANS	Euler-Euler	No	Stirred tank (2 L & 50 L)	2014	[29]
k-ε	Euler-Euler	No	Stirred tank (100 m³)	2017	[30]
k-ε	1 phase Hydro	9-pool metabolism	Stirred tank (3 L & 54 m³)	2017	[31]
LES	1 phase Hydro	No	Stirred tank (20 L)	2010	[32]

As shown in Table 5-4, *Ansys Fluent* is capable of the most simulation methods applied within the in-house developed CFD tool (LES, Biological models, Euler-Lagrange). The lattice-Boltzmann method is widely used in CFD simulations with particles, but no relevant information about an application for bioreactor modelling could be found in scientific literature. The Euler-Lagrange approach in *Ansys Fluent* provides very good

results only if the phenomena of bubble breakage and coalescence are of minor importance [40]. In comparison, the in-house developed CFD tool provides an algorithm where these phenomena are accurately modelled.

Very important for the competitive analysis are the industrial-scale simulations. Wang et. al. and Nauha et. al. present simplified CFD models, which are therefore not comparable [41], [42]. Haringa et. al. applied in a simplified one-phase fluid a complex 9-pool metabolic model for *P. chrysogenum* in a 54 m³ bioreactor [43]. The study of Morchain et. al applies a Euler-Euler approach for the two-phase flow, a PBM for biological adaptation to concentration gradients, and a kinetic model for biological reactions on a 70 m³ bioreactor [44]. This publication proves that *Ansys Fluent* is capable of simulating industrial-scale bioreactors. However, necessary information for a representative comparison like calculation time or set-up time cannot be found.

Comsol Multiphysics

With 1.5% market share and 100,000 users in 2015, *Comsol* is relatively small in comparison to *Ansys* [45]. Their CFD tool *Comsol Multiphysic*s can be extended with different modules and is relatively cheap in comparison to *Ansys Fluent*. A lot of research based on *Comsol Multiphysics* can be found although it has a small customer base. An overview about relevant papers is presented in Table 5-5.

Table 5-5: Relevant CFD models of bioreactors based on *Comsol Multiphysics*

Turbulence	Multiphase Model	Biologics	Reactor Type	Year	Reference
k-ε	Euler-Lagrange	No	Airlift	2015	[46]
-	-	-	Stirred tank	2012	[47]
laminar bubbly flow model	Hadamard–Rybczynski drag law	No	Airlift (15 L)	2015	[48]
-	Euler-Euler	No	Airlift (lab-scale)	2012	[49]

As shown, stirred reactors with multiphase flow can be modelled. No representative information about the application of a biological model and a large eddy turbulence model could be found. Same as *Ansys Fluent*, the LBM is not used for bioreactor modelling. However, *Comsol Multiphysics* is capable of the Euler-Lagrange multiphase model approach. No studies or other representative papers about the modelling of industrial-scale bioreactors could be found.

OpenFoam

OpenFoam is an open source CFD code without an integrated GUI in the original version. However, many papers regarding bioreactor modelling can be found. Table 5-6 shows a selection of relevant studies.

Table 5-6: Relevant CFD models of bioreactors based on *OpenFoam*

Turbulence	Multiphase Model	Biologics	Reactor Type	Year	Reference
RANS	Euler-Euler	No	Stirred tank (<1 m³)	2018	[50]
k-ε	Multiple Reference Frame	No	Airlift	2011	[51]
k-ε	Euler-Euler	ASM1	Activated sludge reactor	2018	[52]

As shown, *OpenFoam* is also used for bioreactor modelling. Biological models can also be considered with this tool. *OpenFoam* is also capable of the Euler-Lagrange approach, the LBM and the LES, but are currently not used in related simulation tasks of bioreactor modelling [53], [54]. Same as *Comsol Multiphysics*, no representative paper about industrial-scale bioreactor modelling can be found.

Aspen

Although *Aspen* (Product: *Aspen One*) is no CFD tool, it is a powerful modelling tool for bioreactors [55]. Additionally, it can be combined with *Ansys Fluent* [56].

M-Star CFD

M-Star CFD is an enterprise in the USA that sells a self-made CFD tool, called *M-Star CFD*. The tool applies the same model approaches as the in-house developed tool (LBM, LES). Additionally, laminar flows are modelled with direct numerical simulation (DNS). The in-house developed tool refrains DNS because it is not feasible for industrial-scale bioreactors due to massive computational effort [4]. However, GPU support of the software is planned in 2018. Like the in-house developed tool, *M-Star CFD* uses *PARAVIEW* for post-processing. Geometry setup can be accomplished with CAD-input, which accelerates setup of complex bioreactors. Currently, biological models cannot be considered with this tool [57].

To sum it up, *Ansys Fluent* is the only tool capable of all functions offered by the in-house developed tool. *Ansys Fluent* is also the only simulation tool, where biological models have been used in a multiphase stirred tank model. However, except for the biological model, *M-Star CFD* has a similar modelling approach as the in-house developed tool. The LBM is rarely used in *Comsol Multiphysics* and *OpenFoam*. No information about computational effort and set-up effort could be found. *OpenFoam* doesn't have a GUI. Therefore, it can be stated that the set-up effort for a bioprocess model takes a lot of time. Meshing of complex geometries can also be time-consuming in the simulation set-up process. However, this task is not necessary for simulation approaches based on the LBM due to constant grid sizes. LES is also only applied in *Ansys Fluent* and *M-Star CFD*. Industrial-scale bioreactors are also only modelled with *Ansys Fluent*. Based on the presented findings it can be stated that *Ansys Fluent* and *M-Star CFD* are the main competitors. To reveal potential advantages or disadvantages of the in-house developed CFD tool, a relative competitive analysis is carried out in the following section.

Relative competitive advantage

The applied method to analyse the relative competitive advantage of a product compared with competitive products is described in [38]. Listing of important criteria of the in-house developed CFD tool is the first step, an unbiased rating of these criteria the second step. Table 5-7 shows the chosen criteria and their rating based on simple grades (1 =

criterion is fulfilled excellent, 5 = criterion is not fulfilled). Focus of the rating is set on modelling industrial-scale bioreactors.

Table 5-7: Rating of the most important criteria of the CFD tool

Criterion	Properties used for rating	Grade
Calculation time	LBM; parcel approach bubbles	1
Range of use	Lab-scale and industrial scale	1
Functionality	Stirrer geometries; pellets; biological models	2
Expandability	Changes/ extensions in source code	3
Handling	Parameter sheet input	2
Post-processing	Visualization and functions	2

After rating the own CFD tool, direct competitors have to be graded in the same way. The result of the competitive comparison is shown in Figure 29.

	Weighting	Ansys		Comsol		OpenFoam		M-Star CFD		In-house CFD	
		G	P	G	P	G	P	G	P	G	P
1. Calculation time	10	2	20	2	20	2	20	2	20	1	10
2. Range of use	10	3	30	3	30	2	20	2	20	1	10
3. Functionality	5	1	5	3	15	1	5	2	10	2	10
4. Expandability	5	1	5	2	10	1	5	3	15	3	15
5. Handling	2	1	2	1	2	3	6	1	2	2	4
6. Post-Processing	5	1	5	1	5	3	15	2	10	2	10
Sum			67		82		71		77		59
Grade		**1,8**		**2,2**		**1,9**		**2,1**		**1,6**	

Figure 29: Rating of competitive CFD tools and the in-house developed CFD tool based on six criteria with different weighting

Based on the selected criteria, weighting and grading, the in-house developed tool receives a better averaged grade than competitive tools. Figure 30 visualizes its relative strengths and weaknesses compared to competitive tools.

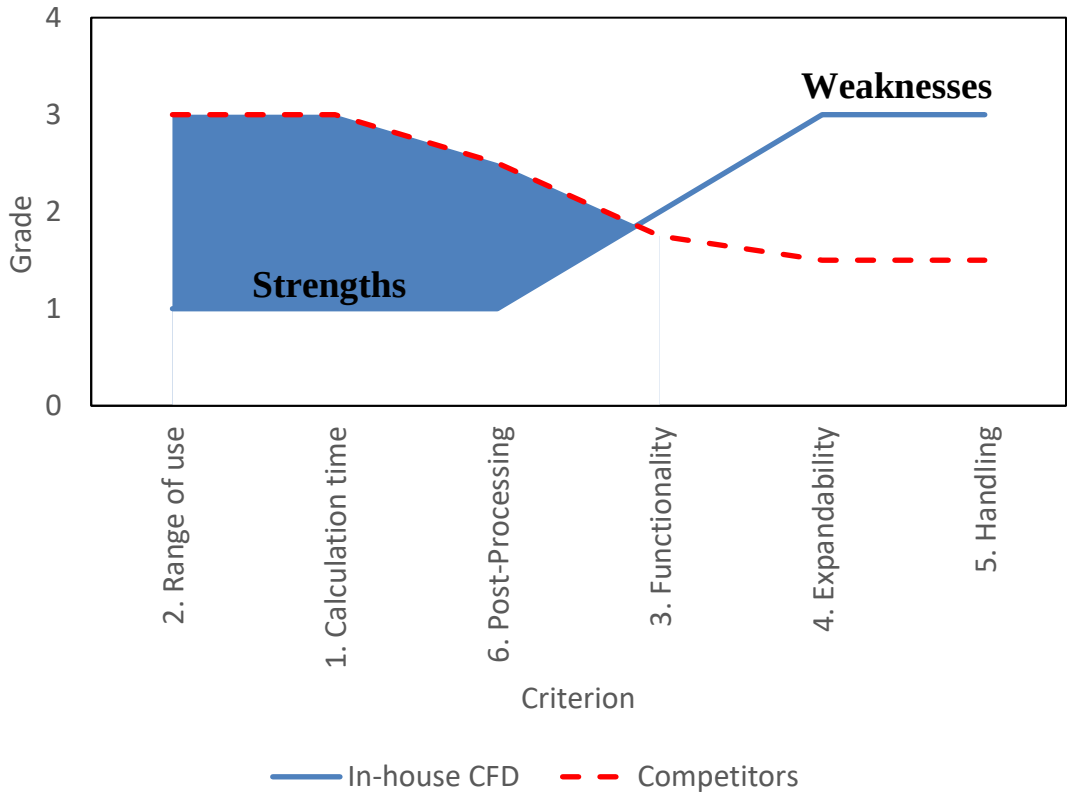

Figure 30: Relative competitive advantage of the in-house developed CFD tool compared to its competitors

The relative competitive advantage in comparison to all considered competitors are its flexibility regarding reactor volume and calculation time. Post-processing is graded similar to competitors due to the powerful tool *PARAVIEW*. Handling and expandability of competitive tools is rated better. Main reason for the advantages of the in-house developed tool is its specialisation on industrial-scale bioreactors while competitive products cover a wider range of application. Fields of application for the in-house developed tool are already presented in Chapter 1. For a further inside into capabilities of the tool, a SWOT analysis is carried out in the next chapter.

6 SWOT-analysis

Goal of a SWOT-analysis is the derivation of strategies based on product strengths, weaknesses as well as external opportunities and threats. The derived strategies have to be considered in a following business model. The SWOT-analysis is very important, because it allows to run different future scenarios. In general, taking advantage of opportunities and strengths while minimizing weaknesses and threats is the result of the strategies derived from the SWOT-analysis [58]. The developed strategies are used for the derivation of a business model with a maximum utilization of the tool's capabilities.

Strengths

The strengths of the in-house developed CFD tool are capabilities that lead to its competitive advantage. For a better overview, all strengths of the tool can be summarized into following three topics and are discussed in the following.

- Application issues
- Code issues
- Environmental issues

Application issues

Applications of the CFD tool are engineering tasks on bioreactors. Due to the limitation on bioreactor modelling, a simple handling of the tool is possible. It provides all necessary capabilities to model a bioreactor, like handling with multiphase, biological models, heat transport and species transport. The simulation setup is based on simple parameter input. In comparison to the complex input and handling of competitive tools, this concept allows a fast setup handling, also for users that have no explicit CFD education. Basic geometry changes like different stirrer types or varying number of baffles can be done easily.

The tool is designed for fast modelling of large bioreactors that can have up to 200 m^3. Due to high effort in reducing the computation time during code development, the calculation of large reactors is very fast compared to competitive products. The use of the tool in one pharma company already proves its practical suitability. During the code development and its application at a pharmaceutical company, the project team also gained a lot of know-how.

Based on this strength analysis, unique selling position (USP) of the in-house developed CFD tool is obviously the fast CFD simulation of industrial-scale bioprocesses, where no CFD specialists are needed.

Code issues

The fully adaptable code is based on *C++*. It is executed on GPUs and CPUs to allow fast calculations. To ensure stability in the team, maintaining as well as developing know-how and constant improvement of the code, all main programmers are financed until end of 2019.

Environmental issues

The code is completely developed at the TU Graz. The university provides a high-tech infrastructure and support for spin-off and legal issues. Worth mentioning are industry contacts of the institute and the *RCPE* as well as available spin-off experience.

Weaknesses

The weaknesses are mainly determined by following issues. Minimizing weaknesses is a task of an appropriate corporate strategy.

- Limitations of the tool
- Software development issues

Limitations of the tool

The main weakness is the restricted field of application. Due its limitation on bioreactors, the market potential in comparison to competitive CFD tools is smaller. The lack of an up-to-date GUI can also be considered as a weakness, because it reduces the user-friendliness and appearance of the tool. A CAD supported input for new geometries is also not implemented. If the desired geometries differ from the available ones, they have to be implemented into the code. Although the extern open source post-processing tool *PARAVIEW* is very powerful and user friendly, extern post-processing can be considered as a weakness due to the dependency of an additional software.

Software development issues

For CFD users, the accuracy of the simulation algorithm is of high importance. Accuracy is guaranteed by a validated tool. However, for industrial-scale bioreactors, hardly any representative data for validation is available. Additionally, validating simulation results is difficult as many processes (parameters, geometries) influence the result. Due to validity and the complex interconnection of functions in the whole code, a test harness is

necessary. It guarantees that all functions of the code work properly by comparing results to pre-defined reference values. This test harness is not finished. After finishing, the test harness becomes a strength.

Furthermore, it is very hard to find talented programmers with simulation background at the TU Graz that can support the project with valuable input. Another problem is the current lack of relationships with companies. The project team have a partnership with one pharma company, which is obviously not enough for a customer base.

Opportunities

With an appropriate corporate strategy, those external factors can be used as a competitive advantage. For the in-house developed tool, the opportunities are based on following issues.

- Unfulfilled needs
- TU Graz aid

Unfulfilled needs

Quality by Design is a fundamental approach in the pharmaceutical industry that caused a drastic improvement of product and process quality. Scale-ups are a critical part during a pharmaceutical process. In need for deeper process understanding, the tool can provide the companies with necessary data that may otherwise require lab experiments [1].

With individual biological models, optimizations based on the actual product output can also be accomplished. A flexible approach of the biologic models can also face engineering tasks for new fields of application (e.g. artificial meat).

The main opportunity, which utilizes the tools unique selling position, is the need for CFD tools capable of modelling industrial-scale bioreactors. Competitors also can handle large scale bioprocesses, but with much longer simulation times. In general, scale-up and scale-down problems of biotechnological companies can be minimized by using appropriate CFD software. To cover a wider range of reactors, perfusion operation of bioreactors should also be able to be simulated.

CFD tools often offer conservative licensing methods, like renting the very expensive license for a year. It is assumed, that many biotechnological companies need a more flexible solution for their engineering problems. It is expected, that a flexible approach of a selling concept will be appreciated by the market.

TU Graz aid

The relationship to the TU Graz offers some significant benefits in supportive aspects. Besides the offered office space, a highly sponsored spin-off fellowship for a successful market launch is also possible. Legal questions may also be supported by the law department of the TU Graz.

Threats

Possible threats for a successful tool can be minimized with an appropriate corporate strategy. Threats can be separated into following topics.

- Market issues
- Technical issues

Market issues

Once the tool is ready for the market, the tool has to deal with several threats. It is assumed that a start for CFD newcomers is very difficult, because the market is highly competitive and dominated by *Ansys*. Besides the market conditions, competitors can copy the tools strengths very fast due to their available personnel and know-how. Before a possible market launch, essential legal questions like the ownership of the code and copyright issues must be clarified.

The pharmaceutical industry is well known for its conservative attitude. Therefore, it can be expected that possible customers show a sceptical behaviour in order to accomplish scale-up tasks with CFD, not with experiments. Additionally, there is a trend of downsizing bioreactors to reduce the facility footprint, as already mentioned in Section 5.2.

Technical issues

Fundamental requirement for a successful market launch of a CFD tool are correct simulation results. Wrong results may lead to a loss of trust for the product. Therefore, in-depth tests are necessary.

Figure 31 presents a summary of the applied SWOT-analysis. As already mentioned, the differentiation between internal factors (SW) and external factors (OT) is very important for the following derivation of a corporate strategy.

Strengths
Fully adaptable code
Fast simulations in comparison to competitors
Applicable on lab-scale and industrial-scale reactors
No CFD specialists needed
All in one tool
Full understanding of code due to in-house development
Experienced due to research project with pharma company

Weaknesses
Difficult validation
Limited GUI & no complete test harness
Currently limited to stirred- and airlift reactors
No CAD input: limited on parameter input
Difficult recruiting
Post-processing not included in the tool
No customer base

Opportunities
Simulations required for Quality by design improvement (QbD)
Modelling single-use bioreactors
Can be used in many industries
Industrial-scale reactors are rarely modelled
Learning from mistakes on the market made by competitors
Spin-off fellowship
CFD specialists are expensive

Threats
Competitors could achieve the software development faster
Ownership of the code unclear
Not all simulation results are validated
Sceptical customers
Difficult start for newcomers on the CFD market
Trend to downsize facilities
Copyright issues

Figure 31: SWOT-analysis of the in-house developed CFD tool

Strategic development

The next step in a SWOT analysis is the TOWS matrix. In this matrix, all founded factors are identified and then paired with the intention of stimulating a new strategic initiative [59]. Figure 32 shows the general approach of a TOWS matrix.

	Strengths	Weaknesses
Opportunities	SO strategies	WO strategies
Threats	ST strategies	WT strategies

Figure 32: Principle of the TOWS matrix for strategy derivation

SO strategies

These kinds of strategies are developed to identify opportunities that are most compatible with the tool's strengths. The most obvious utilization of a strength that also fits the USP is the fast modelling of industrial-scale bioreactors, where no CFD specialists are needed. Due to the fully adaptable code, new biologic models as well as new geometries can be considered. This allows covering many different bioprocesses, as already shown in this thesis. It is assumed that new fields of application of bioreactors e.g. artificial meat is not going to be carried out in large-scale bioreactors, at least in the foreseeable future. Therefore, extension of the biological models for extraordinary biological models is not priority. More important is the need for fast scale-up and scale-down of bioreactors to increase QbD and reduce the need for expensive experiments. Implementation of scale dependent parameter for faster up- and down scaling would therefore utilize opportunities.

To fulfil the industry's need for a flexible applicable CFD tool, an appropriate business model that utilizes strengths of the tool is developed in Section 7.2. The opportunity of a financed spin-off fellowship should also be utilized, to increase quality, validity and accuracy of the code.

ST strategies

Goal of ST strategies are the minimization of external threats by using the tools strengths. Maintaining high confidentiality and a fast market launch minimizes the risk of an anticipation of competitors like Ansys. Before any further effort is invested in a preparation of the tool for a market launch, ownership discussion has to be finished. In order to convince conservative companies for using CFD software, prepared simulation scenarios (scale-ups) and validated results can be presented. Minimizing the threat of

wrong simulation results is also essential. Validation concepts can be developed and offered as student works that main programmer can focus on further code development.
In general, the downsizing of facilities and more frequent use of single-use pharma-bioreactors may be a threat because the competitive advantage of simulating industrial-scale bioreactors gets lost. It is assumed that the downsizing trend develops very slowly, because as already mentioned, the pharmaceutical industry is very conservative. The risk of this threat is therefore assumed acceptable and cannot be overcome by any strength of the in-house developed tool.

WO strategies

WO strategies enables the enterprise to overcome weakness and focus on opportunities. To ensure validated results for QbD tasks, it is recommended that validation and test harness development is carried out for a small range of parameters at first. While the validated range of parameters guarantee an accurate model, simulations without of the range parameters can be used as estimation for decision support until test harness is finished.
The limitation on bioreactors allows the tool to concentrate its effort and resources only on this sector. Status Quo is that the algorithm is capable of LBM, LES, parcel approach for bubbles and the E-L approach. With focusing effort and resources in further developments of bioreactor modelling, the tool can secure its competitive advantage and may become a specialist in this area. Due to high complexity and quality demands of biotechnological processes, it is assumed that a wide acceptance for special tools exist. As already mentioned in Section 1, Quality by Design requirements contribute to the expected increase of simulations in biotechnological industry.
A marketing strategy should be developed, which satisfies the industries need for a flexible demand of simulations. If necessary, the future selling concept should also focus only on a limited GUI to minimize coding effort.

WT strategies

Preventing weaknesses from an exposure to threats is the goal of WT strategies. There is no chance of pushing established competitors out of the biotechnological market with copying their selling concepts and marketing strategy because the tools are not comparable. Instead, a focus on the tools unique selling position while using a different selling concept than competitors is recommended. The new approach for modelling

bioreactors can offer a successful market position, especially if an appropriate selling concept is chosen. Companies that are not convinced by the benefits of CFD may change their opinion for a tool with a flexible selling concept and an easy simulation handling.

The commitment to a business model is related with a compromise, because utilizing all strengths of the tool in one business model is not possible. In the following, different sales formats that are fundamental for a business model are derived. The currently most feasible one is chosen and builds the basis for the following business model.

7 Developing a business model

Results of the market and competitive analysis and the SWOT-analysis influence the development of a business model. Commitment to one business model allows a more detailed definition and analysis of competitors and the market and is also accomplished in this chapter. Based on those analyses, a first business case scenario can be derived and presented. In this analysis, first insights into the marketability are possible.

7.1 Sales format

While commercial CFD tools offer only traditional forms of software licenses, the in-house developed tool has the opportunity to fulfil customer needs in a better way. Based on findings of the SWOT analysis, the tool should offer a flexible selling concept where additional coding effort due to GUIs or program framework should be minimized. In this chapter, several concepts are introduced and discussed.

I. Sales format – Capsule

This concept is based on the capsule's sales format of *Nespresso*. One capsule is a recurrence CFD base data module for one reactor geometry, one stirrer speed, one fluid property set, one biological model and one gas flow rate. For further geometries, properties and so on, the customer has to buy additional capsules. Different capsules can also be combined in one simulation (fluid properties are changing during the simulation). However, this concept requires a GPU-Server for the customer.

The capsule concept provides a flexible CFD software, where customers can extend the tool according to their needs. Due to the concept, code manipulation for the customers is impossible and therefore a stable program is guaranteed. A GUI would also be easy to implement, because the simulation requires only the selection of capsules and the duration. However, a significant coding effort is necessary to obtain a user-friendly software (installation, GUI, code to application). Therefore, this concept is currently not realizable.

II. Sales format – Service provider

As a service provider, the enterprise would simulate and optimize engineering problems in house. The main advantage of this concept is that customers have no contact with the

tool. Installation issues, GUI development and time-consuming customer support can be avoided. The whole Know-How also remains in the development team. Similar projects can be accomplished with less effort, because of similar parameters and procedure. On the other hand, the part the USP "no CFD specialists needed" gets lost.

Important for the clients is the flexibility of this concept. They don't have to purchase a full CFD tool although they may need it rarely. Additionally, they don't need a GPU-server for faster calculations. In this concept, customers would assign the service provider for clear defined optimization and engineering tasks.

From the present point of view, this concept is the fastest one to accomplish because of the low additional coding effort. Also, Know-How and calculation power for fast project handling is available at the TU Graz.

Outsourcing study

A study from 2008 reveals the key factors of R&D outsourcing in the UK pharmaceutical industry [60]. According to them, reasons for outsourcing can be grouped into overall push and pull factors (initiating factors) and framing factors which shape and delimit the nature and type of activities accessed externally. While initiating factors contain factors like cost and time savings, framing factors can include degrees of uncertainty, regulatory constraints and the ability to reabsorb research and knowledge. According to the study, the four most important outsourcing factors are:

- Access expertise not available in-house
- Reduce development time and time to market
- Reduce development costs
- Support for technology change in process

They also listed the barriers for external innovation sourcing according to their importance. The most important four factors are:

- Confidence in the contractor/ partner's ability to deliver relevant solution
- Concern that key knowledge/intellectual property will leak out to contractor/ partner
- Concern that research/ technology too central to firm's competitive advantage
- Regulatory restrictions on outsourcing

It is obvious that main concerns for outsourcing are determined by a loss of internal know-how. This is also a finding of the study. A concluding remark of the survey is that included companies seem to have focused their outsourcing activities on the more peripheral, non-core research and technical activities. However, a quarter of overall pharmaceutical R&D spending in Britain was outsourced in 2005 [60].

The listed barriers increase the difficulty for a successful acceptance as a service provider, because fermentation conditions, reactor geometry and fluid parameters are considered as the heart of every bioprocess. Thus, special focus of a service provider in the pharmaceutical sector should be on realizing this desire. Of course, developing instruments and methods to deal with this issue are necessary for successful venture.

III. Sales format – Classical model

This concept is based on a classical software purchase. After payment or subscription, customers will get access to the tool and support. Needed GPU-Server can be offered as a turnkey solution with installed software. Disadvantage of this concept is the enormous coding effort for update service, GUI, stability and user friendliness. Additionally, a necessary flexible selling concept is not provided with this strategy. Therefore, a further development based on this concept is not recommended.

IV. Sales format – Cloud based model

With a cloud-based GPU server, GPU servers on customer side are not necessary. For example, online GPU access is offered by amazon and can be rent per hour. Due to container platforms (*NVIDIA* – Docker), installing and set-up of the in-house developed CFD tool on GPU-server can be accomplished easily and fast from anywhere. Table 7-1 shows three different GPU server classes of amazon and prices for renting.

Table 7-1: Overview of *Amazon* EC2 P3 instances [61]

Instance size	GPUs Tesla V100	Memory (GB)	Price per hour ($)
p3.2xlarge	1	61	3.06
p3.8xlarge	4	244	12.24
p3.16xlarge	8	488	24.48

As shown, the prices are very low in respect to an equivalent GPU server. A flexible licensing method can also be guaranteed with this concept. It provides a system, where installation, support and GPU server management can be done in house. In comparison to the previous discussed service concept, this concept utilizes the tool's strength "No CFD specialists needed" and customers do not have to share confidential information, because they use the tool on their own.

However, this selling concept is currently not realizable due to massive coding effort for a GUI and for a user-friendly application. The focus is currently on improving the functionality of the tool and not on developing a user-friendly application. But with further code development, this concept provides an alternative strategy for the selling concept and should be focused in the next years.

Conclusion

To sum it up, the sales strategy of a CFD service provider is currently the most realistic one due to a manageable additional coding effort. Providing simulation know-how is also one of the strengths of the development team, which is a main reason for outsourcing R&D tasks. Trust concerns of clients regarding knowledge transfer needs to be addressed and minimized. However, it can be expected that the enormous benefit for pharma companies convince possible customers to outsource simulation tasks. An additional hurdle of this selling concept is that many bioreactor manufacturers like *ZETA* already simulate and model their reactors in house to increase quality and competitiveness [62]. As a service provider, bioreactor manufacturer should also be considered in the target market.

The cloud-based and capsule concepts provide a good alternative with full utilization of the tools strengths but are currently not realizable due to necessary code development. Resources from a possible funding (spin-off fellowship) are mainly needed for validation and extension of the tool as well as clarification of legal questions.

7.2 Business model

The next logical step after setting up the business concept is the development of a business model. According to Morrisa, Schindehutte and Allen, a business model can be described as an architecture, design, pattern, plan, method, assumption, and statement of an

enterprise where the level of detail increases from the economic to the operational to the strategic levels [63].

The St. Gallen Business Model Navigator provides 55 business model patterns that are the basic principles of every business model. As a CFD service provider, the potential enterprise can be assigned and named as a *Solution Provider*. According to the St. Gallen Business Model Navigator, future competition is based on business models, not products and technologies [64]. Therefore, flexibility in a business needs to be considered too. Table 7-2 shows a summary of the St. Gallen Business Model Navigator, where the term *Solution Provider* is explained in detail.

Table 7-2: Fundamentals of the concept *Solution Provider* [64]

St. Gallen Business Model Navigator: Solution Provider
Offering total coverage of services and products in a particular sector
Know-how transfer to the customer leads to an increase of his or her efficiency
Improving services based on insights of a close customer – company relationship

Although this general pattern describes the business model very well, a more detailed explanation is necessary. A detailed framework for business model derivation is presented in the publication “The entrepreneur’s business model: toward a unified perspective” [63]. It consists of three levels and six basic decision areas. The first level is called foundation level. At this level, basic decisions about the business are fixed. All enterprises have to make the same decisions on this level. Therefore, the model proposes different choices on the foundation level. The next level, the proprietary level is a more specific definition of the corresponding operation rule. The focus lies on the creation of value in each of the six decision areas. Operating rules are the third level. They execute the decisions set in level two and ensure a framework and guideline in business operations.
Figure 33 shows the derived business model for the *Solution Provider*. In its essence, the model relies on the capabilities of the in-house developed tool. As already mentioned, the novel simulation approaches for fast modelling of industrial-scale bioreactors is the main strength and needs to be utilized as shown in the proprietary level. Operating rules are

currently not executed in detail and are meant to give an idea about their meaning and a rough direction for the *Solution Provider*. More detailed operating rules are necessary in the development of a specific business plan. However, the applied concept allows a detailed characterization of corporate strategy and a rough direction on the market.

Technical, competitive and strategic aspects of the tool have already been explained in detail in previous chapter. However, economical aspects are also important for a venture and are therefore discussed in the following section. As an IT service provider, the enterprise is expected to have high fixed costs compared to its variable costs. Fixed costs are constant and independent of sales volume. Expenditures like personnel or office rents can be assigned to fix costs. In comparison, variable costs like extra work or flexible equipment renting are expected to be lower. Therefore, the *Solution Provider* shows a high operating leverage. This means that sales are a must to cover fixed costs. Due to the restricted resources, only a low number of sales are possible, at least in the beginning phase, and a high price is necessary. However, the overall costs for simulation tasks is expected to be lower than competitor's ones because no expensive licenses for CFD tools like Ansys are needed. Thus, a higher margin is allowed. Margin is the difference between the sales price and the overall costs of the CFD service. A detailed cost estimation is shown later in Section 7.4.

Business Model: Solution Provider			
	Foundation Level	**Proprietary Level**	**Operating Rules**
Component 1: Factors related to offering	CFD service only	Easy set-up, fast handling	Maximum effort for code adaption __h; Maximum computation time due to GPU-Server occupation __h;
	High customization	Adaption to customer's reactor	
	Specialized on a small field	Stirred/ Airlift reactor only	
	Complete solutions	Full process modelled in detail	
	Sell service by itself	No commissioning, measurements	
	Internal service delivery	Direct contact to customers	
	Direct distribution	Costs based on effort estimation	
Component 2: Market factors	Business-to-business	Customers are companies	Offering competitive engineering solutions based on high-tech CFD tool; Addressing trust concerns;
	International	Worldwide customers	
	Retail	Selling solutions to clients	
	Niche market	Focused on bioreactor modelling	
	Relational	Cultivate customer-relationships	
Component 3: Internal capability factors	Offered technology	Novel modelling approaches	Detailed problem outline with customers; Maximum __ projects per month to gather focus; Validation of new functions;
	Experience	Confirmed applicability	
	Fixed costs	No expensive licenses needed	
	Personnel	No CFD specialists needed	
	Time management	Fast computations	
	Supportive environment	TU Graz resources/ know-how	
	Code development	Full adaptable code	
Component 4: Competitive strategy factors	Image of operational excellence	Experienced team	Novel engineering solutions that minimizes lab experiments of the customers;
	Image of operational speed	Fast and easy modelling	
	Innovation leadership	Flexible outsourcing possible	
	Adaptable	Code adaptions improves tool	
Component 5: Economic factors	Flexible pricing and revenue	Flexibility with customers	Fixed costs of enterprise should be below ___€ in the first two years to minimize risks and maintain flexibility;
	Operating leverage: high	High fixed and low variable costs	
	Volumes: low	Manageable number of projects	
	Margins: high	High-tech engineering service	
	Support	Funding for start-ups	
Component 6: Growth factors	Growth model	Constant growth	Growth after market acceptance;
	Risk acceptance	Minimizing risks	

Figure 33: Derived business model consisting of three levels and six decision areas

As a *Solution Provider*, the presented business model is developed to utilize the tools strengths. With the now available business model, a more concrete view on the *Solution Provider* is enabled. As already mentioned, a business model is very flexible and can change during time. Therefore, changes of the business model until a venture is realized can be expected.

7.3 Market analysis of the Solution Provider

Overview of the consulting market

In the biotechnology industry, outsourcing is very common due to a higher need in flexibility and necessary development time on the market [60]. The main benefits and concerns in outsourcing are already listed in Section 7.1. According to a biotechnology report [65], about 20% of all outsourcing spending is invested in consulting. This ratio is expected to stay constant. In the report, an increase of the outsourcing spending of about 250% in 2025 with respect to 2014 is predicted. Spending in consulting is also expected to rise with the same ratio. In 2015, the outsourcing volume of the U.S. biotechnology industry is declared with 19.4 billion US$ [65] while the overall revenue was 107.4 billion US$ [66]. Thus, the growing consulting market in the global biotechnological sector can be assumed as very large.

Exemplary project calculation

As an engineering consulter, the *Solution Provider*'s payment depends on the needed working hours. Oriented at typical hourly prices for CFD consulting of about 100-150$, the hourly price is assumed with 125€ [67]. The number of working hours depends heavily on the project size and properties. Additionally, industrial-scale bioreactors have a high customization due to the wide range of products, environmental conditions and quality requirements. In Table 7-3, a breakdown of a standard project's working effort of the *Solution Provider* is listed. This standard project is a reactor optimization, where the goal is to find the best process parameter for a high product output. It is assumed that no significant code adaption is necessary for the exemplary standard project.

Table 7-3: Time breakdown of a standard project for the *Solution Provider*

Working time	Corresponding tasks
5h	General project configuration (i.e. biologics, fluid)
7x5h	Configuration of cases (i.e. Stirrer, heat exchanger, oxygen)
5h	Simulation set up
30h	Post processing and evaluation
5h	Documentation
80 h	Overall effort

Simulation time is considered with a flat rate of 2,500 € in the standard project. With the previous defined hourly price of 125€, it can be assumed that a standard project generates a revenue of 12,500 €. Of course, projects that do not fit in the standard project specification needs a new cost evaluation due to the necessary additional coding effort or simulation time.

Detailed look on customers of a Solution Provider

Possible customers for a *Solution Provider* with specialization on bioreactor engineering are companies which uses or produces stirred or airlift bioreactors. Main focus is on the pharmaceutical industry due to their need for process optimization, deeper process understanding and expected growth. In Table 7-4, possible customers in the focused pharmaceutical industry sorted to their profession are listed.

Table 7-4: Exemplary customers in the pharmaceutical industry

Bioreactor manufacturer	Pharma company	CMO
Zeta	*Novartis*	*Lonza*
Bilfinger	*Pfizer*	*Samsung-Biologics*

After presenting the effort and revenue of an exemplary project, a detailed analysis of on possible customer can be made. This analysis enables an estimation about the possible

revenue generated with one customer. Selected customer for this analysis is the Swiss contract manufacturing organization (CMO) *Lonza*. CMOs offer capacity for different production steps and experienced an enormous growth over the last years. The average booking time of CMO capacity is 16 months [68].

Due to the legal form of a stock corporation, capacity and production data of *Lonza* are open accessible. *Lonza's* bioreactor capacities can be used for the fermentation of mammalian cells and microbial fermentation. Table 7-5 presents a detailed breakdown of *Lonza's* bioreactor capacity, distributed over the whole world.

Table 7-5: Breakdown of *Lonza's* bioreactor capacity [69], [70]

Fermentation type	**Location**	**Reactor Capacity**
Mammalian cells	Tuas, Singapore	4 x 20,000 L
	Portsmouth, USA	3x 5,000 L; 4x 20,000 L
	Porrino, Spain	4x10,000 L
Microbial	Kourim, Czech Rep.	5x 15,000 L; 5x 50,000 L; 2x 75,000 L

Additionally, *Lonza's* annual report of 2017 states that the CMO supplied 50 commercial medicines. 22 of the 50 supplied medicines are produced with mammalian cells. From the remaining 28 medicines, it is assumed that 10 are produced with microbial fermentation, the rest are fine chemicals [71]. To sum it up, it is assumed that *Lonza* produced 32 biopharmaceutical medicines in 2017.

32 different biopharmaceutical products mean 32 different bioreactor runs with different process conditions and parameter. Every different bioprocess carries the potential for a demand of the *Solution Provider* because scale ups, reactor optimizations or derivation management may be necessary. Applying the previous determined standard project revenue of 12,500 €, a customer like *Lonza* carries a potential annual revenue of 400,000 € in 16 months. In comparison, the pharmaceutical companies *AstraZeneca* and *Roche* each accomplished two *FDA* approvals for biological based drugs [72]. Based on the two new products, it is assumed that each of the new products caused the development of a new bioprocess last year.

Market potential

The theoretical possible market size is called market potential. It is an important parameter of the target market. Expected trends and growth of the biotechnological and CFD market are also applicable on the market potential. The market potential for a *Solution Provider* is based on Equation 7-1 [73].

$$market\ potential = \#customers * service\ frequency\ per\ time * price \quad (7\text{-}1)$$

An accurate determination of the market potential is very difficult, because all possible customers have to be known as well as their potential demand for a service. However, a rough estimation of the market potential based on the previous determined customers and demand for bioreactor engineering tasks is possible. As already mentioned, possible customers are mainly in the pharmaceutical industry due to its demand and high customization. For the market potential, 10% of all European biotechnological companies (2,259) consisting of bioreactor manufacturer, pharmaceutical manufacturer and CMO's are assumed to need the offered CFD services for industrial-scale bioreactors. The average demand for services is assumed to be 10 times per year. This assumption is based on the previous determined numbers of new processes of three biotechnological companies as well as the need for continuous optimization of already existing processes. The resulting market potential for Europe is shown in Equation 7-2.

$$market\ potential = 225 * 10 * 12{,}500 = 28{,}125{,}000\ €/a \quad (7\text{-}2)$$

Of course, not the whole market potential can be utilized. Hurdles are already mentioned in the SWOT-analysis and in the business model, presented in Chapter 6 and 7, respectively.

Based on the presented difficulties, a potential annual revenue of 450,000 € during the first years can be expected if the *Solution Provider* utilizes its strengths and establishes itself on the market. An additional beneficial factor is the expected growth of the biotechnological and CFD market and in further consequence, the market potential. With an increasing usage of CFD simulations, a *Solution Provider* in this sector is also expected to gain further acceptance.

7.4 Business case of the Solution Provider

With the now available numbers of the previous section, a business case of a hypothetical *Solution Provider* specialized on bioreactor engineering can be carried out. Goal is to list realistic expenses and receipts of a company that works with the considered bioprocess simulation tool.

Financing spin-off

Fortunately, Austria have a founder-friendly environment. A spin-off fellowship offered by *FFG* (Österreichische Forschungsförderungsgesellschaft) seems to be the best solution for financing the project until marketability. The maximum funding is 500,000 €, the duration at least 12 months and at maximum 18 months. Fundable costs are personnel costs, material costs, costs of asset utilization, third-party charges and travel costs. Purchase of equipment is not possible within the spin-off fellowship. However, the usage of equipment is sponsored. To utilize the funding's full potential, GPU-Server (if the existing ones cannot be used) have to be rent or leased. Goal of the spin-off fellowship is the further development of intellectual property so that a following foundation of an enterprise is possible. [74]

Cost and financial estimation after spin-off

This cost estimation enables an overview about accruing costs during the start-up phase. In the course of this master thesis, a general market overview and a conceptual business model is derived. No specific decisions about a market entry are made. For the cost estimation, the most plausible scenario after the spin-off fellowship is assumed. After the spin-off, it is assumed that the TU Graz's equipment including GPU-Servers as well as facilities cannot be used. The free facility and infrastructure of the Science Park Graz is considered as an appropriate alternative. Internet, PC's and general office equipment are offered at this sponsored institution. However, expensive computational power in form of GPU-server is not provided. For the start-up phase, an investment in GPU-server of about 12,500 € is assumed (Capital expenditures – CAPEX). Due to legal reasons, a GmbH founding is necessary and costs about 7,500 € [75]. A further payment for the capital contribution has to be considered. Marketing in the starting phase of a venture is also very important to gain customer attention. A starting marketing budget of 10,000 € is assumed. Fix costs in a future venture consists mainly of personnel costs, because no

expensive CFD software is needed. For the personnel, two PhD with a monthly pre-tax salary of 3,500 € are assumed. With additional fees, the overall personnel costs are about 11,000 € for two employees. The costs for three employees for a further growth are assumed with 16,000. 3,000 € additional budget per month is assumed for different expenses like travel costs, further marketing, capital contribution and extern consulting. Table 7-6 summarizes the estimated fix costs as well as periodical costs (operational expense - OPEX) for the starting phase of the scenario *Solution Provider*.

Table 7-6: Assumed costs of the enterprise

Initial costs		**Monthly fix costs (OPEX)**	
Description	**Costs in €**	**Description**	**Costs in €**
GPU-server (CAPEX)	12,500	Personnel (2x)	11,000
Marketing	10,000	Personnel (3x)	16,000
GmbH founding	7,500	Add. costs	3,000
Sum	30,000	Sum	14,000/ 19,000

To cover all costs of the first operational quarter, capital of 30.000 € is necessary. Additionally, capital reserves are needed to cover OPEX at the beginning of the operational activities. A minimum starting capital of 60,000 € seems appropriate. To cover these costs, the *FFG* start-up funding "MARKT.START" is assumed [76]. "MARKT.START" offers the opportunity of a cheap credit if all requirements can be fulfilled. Payment conditions for the funding are listed in the following:

- 20% own capital quota of the whole funding is needed
- First payment is 30% of the whole funding
- Runtime is limited with 36 months
- Last payment is 10% of the whole funding
- Back payment due of the loan is 5 years after runtime end
- Partial payments are flexible
- Interest are based on the European reference interest rate

Figure 34 presents an overview of *FFG*'s "MARKT.START" funding requirements on the offered product by a venture and corresponding assumptions of the considered business case as a *Solution Provider*.

Funding requirements	Corresponding assumptions for the scenario
A previous founded *FFG* project is finished	Approved and finished *FFG* spin-off fellowship
The product is ready for market	No open challenges and a ready product
Technological advantages in respect to competitors	Clear and obvious unique selling position
Market knowledge and experience	Detailed target market and competitive analysis as well as representation of the market position available
Market expectations	Competitors allow market opportunities

Figure 34: FFG's "MARKT.START" funding requirements and corresponding assumptions for the business case

Based on the previous listed payment conditions of the funding, a more specific overview about necessary and available capital in the starting phase can be carried out, as shown in Table 7-7.

Table 7-7: Assumed initial costs for the estimated business case

Needed capital		**Available capital**	
Description	**Expenses in €**	**Description**	**Capital in €**
Initial costs	30,000	1st *FFG* payment	24,000
OPEX reserve	30,000	Own capital	36,000
Sum	60,000	Sum	60,000

As shown, 24,000 € of starting capital provided by the funding are necessary. Due to the funding's initial payment of 30%, the whole necessary funding is about 80,000 €. Capital provided by the funding is outside capital. Thus, own capital of 36,000 € is necessary. This own capital can be provided by the founders and by investors. Capital provided by

investors is assumed to be repaid with a bonus after a successful establishment on the market.

Based on the presented financing conditions, a finance plan over the first 36 months is estimated. In this estimation, interest is not considered because of the current low interest rate. A sale tax on earnings is considered with 20%. Initial difficulties are also considered with a low revenue in the first months. Table 7-6 summarizes the estimated not operational financial actions of the first 36 months, Figure 35 the corresponding comparison of annual net incomes (incl. funding) and outgoings for the first operational years. The whole estimation for the business case's first 36 operational months is presented in the appendix in Table 10-4.

Table 7-8: Major spending and funding inflow of the *Solution Provider*

	Investments/ spending		**Not operational inflow**	
Month	**Expenses in €**	**Description**	**Inflow in €**	**Description**
0	30,000	Costs acc. to Table 7-6	60,000	Starting capital
6	3,500	GPU-server purchase	24,000	2nd *FFG* payment
20	10,000	GPU-server purchase		
24	40,000	Back payment to investors	24,000	3rd *FFG* payment
36	25,000	IT purchase/ relocation	8,000	4th *FFG* payment

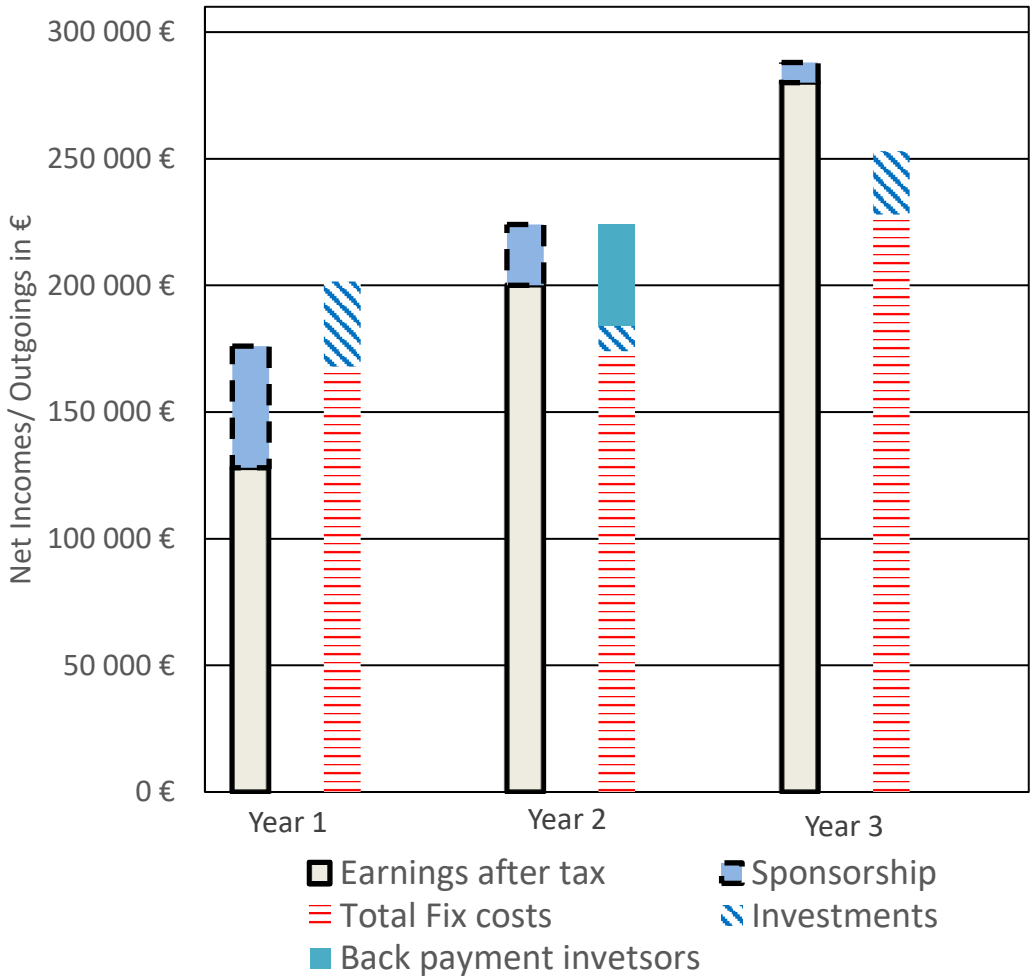

Figure 35: Assumed after tax incomes and expenses of year 1 to year 3. The left pillar represents incomes (earnings after tax and funding), the right pillar expenses (fix costs, investments and back payment to investors)

Due to the *FFG* funding, the *Solution Provider* can cover its own costs after 12 months in business which is shown with positive net receipts in Table 10-4. With the support of a third employee in the third year, the earnings are expected to rise significantly, and a more rapid growth can be assumed so that the enterprise can expand after the fourth year. The back payment of the *FFG* loan must be considered in further financial planning.

The pre-tax revenues should be targeted with 150,000 € in year 1, 250,000 € in year 2 and 350,000 € in year 3. The net receipts of the three years including funding is shown in Figure 35. In the first year, net incomes are significantly lower than outgoings. In this

phase, own capital and the funding are needed to maintain solvency. During the first year, investments are mainly planned directly after founding. In the second year, net incomes should be equal to all outgoings. At the end of the second year, back payment of investor's capital including bonus is considered with 40,000 €. Monthly earnings are high enough that a third employee can be recruited. In the third year, net income is significantly higher than expenses, the enterprise makes profit. Preparations and savings for further expansion and leaving the sponsored office can be considered. A positive financial balance (cumulative receipts) is very important to maintain solvency. The presented financial plan depends heavily on the selected startup funding. During the spin-off, an appropriate startup funding should be chosen. Focus should be on utilizing as much funding as possible.

8 Marketing strategy

The next step after the market analysis, the SWOT-analysis and the development of a business model, is the development of an appropriate marketing strategy. The derived marketing strategy is based on the book “Marketing plans” [77]. Overall goal of the strategy is the definition of future development of the *Solution Provider* scenario and how this growth can be achieved. First part is a unit mission statement, where the framework of an enterprise is described. This is followed by a summary of planned and expected financial future and a market overview that describes the target market. Based on the derived business model, a portfolio of the assumed enterprise and of competitors with similar business models is accomplished. Afterwards, the final marketing objectives and strategies can be defined.

Unit mission statement

The enterprise can be defined as a *Solution Provider* for the biotechnological industry. For this industry, engineering solutions for different kinds of bioreactors are offered.

Customers benefit from CFD simulation supported services and several years of simulation experience in the pharmaceutical industry. Offered solutions minimize the number of needed experiments drastically and enhance process understanding and thus, QbD. Therefore, the venture contributes in a reduction of costs in research and development departments as well as in a reduction of the ecological footprint.

Offered services address multiple problems in the biotechnological industry. Some of them include scale-up, scale-down, reactor optimization as well as stirrer optimization. Besides the experience of the team, solutions are based on an in-house developed CFD tool with novel simulation approaches. Main benefits are the fast simulation of industrial-scale bioreactors as well as the simple set-up and appliance of the tool.

All forecasts expect a further growth of the biotechnological sector. In the future, further development of the CFD tool is planned to utilize the higher demand for CFD simulations. Multiple biological models as well as expanding the palette of reactor types are planned. A market launch of the developed CFD tool might be an option, if the code development continuous to show good progress. To avoid direct competitions in special fields of commercial CFD tools, offering services besides bioreactor engineering is no option.

Summary of financial projections

Essential part of a marketing strategy is an estimation about the revenue development. An estimation about the first three operational years is already discussed in Section 7.4. However, the revenue (pre-tax earnings) is important for the marketing strategy and discussed here in detail.

Figure 36 shows the revenue forecast over three years. For simplification reasons, the targeted revenue per year is presented.

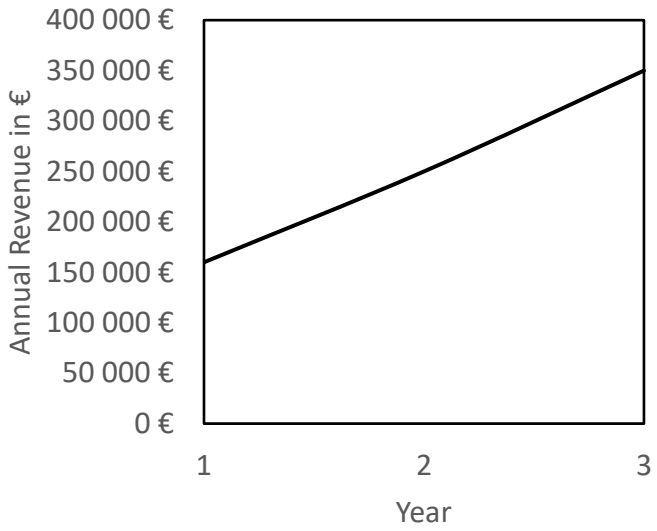

Figure 36: Assumed annual revenue development of the Solution Provider for three years

In the first year, the assumed revenue is about 150,000 €. As shown, the assumed revenue grows to 250,000 € in the second operational year and reaches an annual revenue of 350,000 € after the third year. The purpose of this strategic marketing plan is how these increases will be achieved.

Market overview

A detailed market overview about the CFD market and competitive products can be found in Section 5.3. However, the most important facts are summarized in the marketing strategy. For a better overview of the segmented and analyzed market, Figure 37 presents a market map based on the idea of [77]. The map summarizes the target market of the *Solution Provider*. Competitors work either with commercial tools or open source tools. Therefore, they are represented with these CFD tools. Blue lines describe the marketing

way of the enterprise. Two colored needs are fulfilled by all market participants. The blue ones only by the analyzed *Solution Provider*.

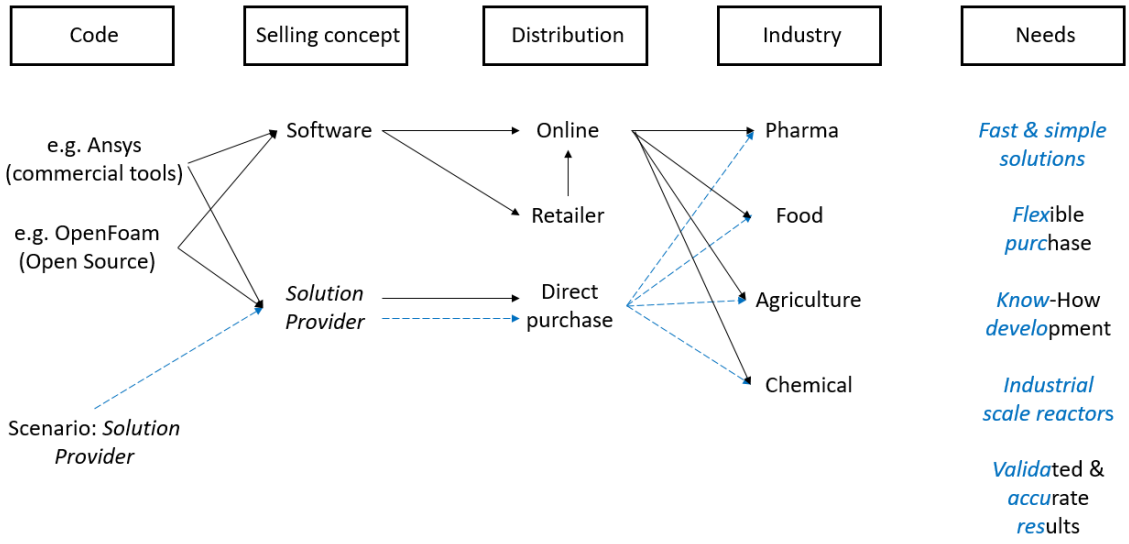

Figure 37: Market map for the Solution Provider (represented with dashed lines). The need "fast and simple simulations" is fulfilled only by the Solution Provider

Portfolio summary of the SWOTs

Goal of the portfolio is a simple presentation of the enterprise's competitive situation. It is assumed that the enterprise is already one year on the market and established successfully. A detailed SWOT-analysis about the developed CFD tool and competitive products is already carried out in Section 6. In this section, a portfolio SWOT summary of the analyzed *Solution Provider* and competitive *Solution Provider* s is carried out. Due to a lack of public information of competitors, this analysis is based on assumptions and estimations. Table 8-1 provides a selection of direct competitors as a *Solution Provider*.

Table 8-1: Possible competitors as a *Solution Provider*

Company	Founding Year	Location	Main Software
SES-Tec	2013	Austria / Graz	*OpenFoam*
SIMTEC	2001	Greece/ SE-Europe	*Ansys*
SimuTech Group	2008	USA	*Ansys*

After defining the competitors, the next step for the portfolio is the definition of market attractiveness and product strength. Figure 38 shows the executed rating based on market attractiveness and product strength.

Classification		Market attractiveness			Sum ideal %	Product strength			Sum ideal %
		Market growth	Industry margin	Pricing		Market share	Profit margin	Technical lead	
In-house CFD	Weighting	2,0	1,5	1,0	9,3	1,5	1,5	2,0	9,5
	Points	2,5	1,5	2,0	13,5	0,5	2,5	2,5	13,5
	Value in use	5,0	2,3	2,0	68,5	0,8	3,8	5,0	70,4
SES-Tec	Weighting	2,0	1,5	1,0	8,3	1,5	1,5	2,0	8,5
	Points	2,0	1,5	2,0	13,5	0,5	2,5	2,0	13,5
	Value in use	4,0	2,3	2,0	61,1	0,8	3,8	4,0	63,0
SIMTEC	Weighting	2,0	1,5	1,0	7,8	1,5	1,5	2,0	7,8
	Points	2,0	1,5	1,5	13,5	1,0	1,5	2,0	13,5
	Value in use	4,0	2,3	1,5	57,4	1,5	2,3	4,0	57,4
SimuTech	Weighting	2,0	1,5	1,0	7,8	1,5	1,5	2,0	8,5
	Points	2,0	1,5	1,5	13,5	1,5	1,5	2,0	13,5
	Value in use	4,0	2,3	1,5	57,4	2,3	2,3	4,0	63,0

Figure 38: Rating of market attractiveness and product strength of the Solution Provider and three competitors in this market

In the section market attractiveness, ratings are almost the same. For the enterprise scenario, market growth is rated with 2.5 due to the strong growth of the biotechnological sector, where its focus lies. The price development of the analyzed *Solution Provider* and *SES-Tec* is rated better, because they use their own software or open source software.

Product strength is mainly determined by technical lead. In the biotechnological sector it is assumed, that the considered enterprise offers the most competetive tool on the market which results in the highest rating of technical lead. The profit margin of the analyzed *Solution Provider* and *SES-Tec* is assumed to be the highest because they do not need expensive software. The market shares of *SimuTech* and *SIMTEC* are assumed higher than the shares of the remaining ones.

The result of the analysis is visualized in Figure 39. The bubble size is based on the estimated revenue size. As shown, the *Solution Provider* is leader in product strength and market attractiveness due to its technical leadership and focus on the fast-growing biotechnological market. The other companies have a heavier impact on the market due to their size and acceptance on the market.

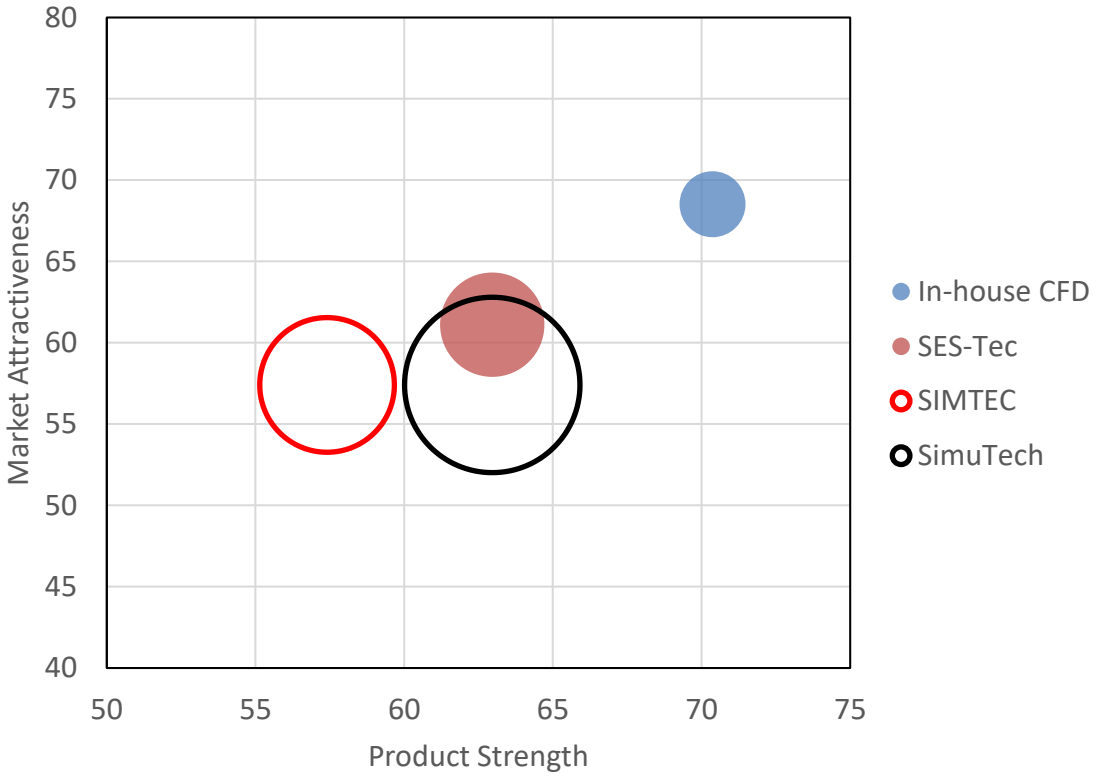

Figure 39: Market attractiveness and product strength portfolio of the Solution Provider and three competitors in this market

Assumptions

Several assumptions are necessary for the scenario of a successful market launch. Assumptions can be considered as unresolved issues for a possible market launch. The most important one is the assumption of clarified legal aspects. In particular, the ownership of the code is important. Additionally, it is assumed that the sponsored spin-off fellowship is approved, which enables a further development of the code and the first contacts to industry. To minimize fix costs, free facilities and infrastructure by the Science Park Graz are assumed. Acquisition costs for GPU-Server are assumed to be covered by the start-up funding "MARKT.START" by *FFG*. Due to the novel modelling approach of the in-house developed tool, a successful starting phase of the enterprise is assumed.

Marketing objectives and strategies for the next three to five years

In general, a marketing objective can be described as the quantification of what an organization sells and to whom and is defined in the marketing strategy. Based on the previous SWOT portfolio, the *Solution Provider* shows high product strength and high

market attractiveness. “Marketing plans” list different strategies for companies with a similar portfolio [77]. According to the book, an appropriate marketing concept can be summarized under the heading *Invest for growth*.

Applied on the *Solution Provider*, focus on a constant further development of the CFD tool is necessary. This can be achieved by accepting complicated projects, where extensions of the code are needed. After several projects it can be assumed, that the in-house developed CFD tool has gained a lot of new functions and possibilities (e.g. set-up and post processing). Additionally, an aggressive marketing posture is recommended. In the following, a principal marketing plan is developed.

Table 8-2 shows a summary of basic revenue objectives, Table 8-3 the respective superordinate marketing strategies. For a better overview, marketing objectives are broken down into the target market segments. In the starting phase, main focus is on biopharma processes due to the available knowledge in this area. However, settling down in the agricultural sector is also recommended, especially in the field of L-Lysine production. The objective after three operational years is that all target markets are opened up.

Table 8-2: Listed revenue goals of the enterprise

Key market segments	**Start-Up Phase in €**	**Planning period t+3 in €**
Biopharmaceutical	135,000	280,000
Food	0	20,000
Agricultural	12,500	30,000
Chemical Industry	12,500	20,000
Target revenue	160,000	350,000

Table 8-3: Principal 4 P marketing strategies

Strategy	Description	Implementation of the strategies
Product	Increase service quality	Taking time for quality improvement Implementing experiences from first projects
Price	Flexible terms/ contracts	Personalized contracts Compromises
Promotion	Focused advertising	7,500 € investment in start-up Continuous investments
Place	Customer integration	2,500 € backup for meetings on site Intense contact during engineering

Principal marketing strategies are derived from the 4P's Product, Price, Promotion and Place. These principal marketing strategies are fundamental for the underlying marketing plan, explained in detail in the following. For marketing, 10,000 € are needed for the starting phase and 750 € per month in the first year.

Due to the small and specialized business, the enterprise relies on a personal communication atmosphere with customers. Benefits are, that personal relationships with customers are enhanced and the intense exchange of information, necessary for a successful solution, is easier. This concept is expensive due to traveling costs. Therefore, financial resources have to be reserved already at the market launch of the enterprise.

Due to the complexity, price and necessary trust of bioreactor CFD simulations, it can be assumed that the first sales need a lot of time and customers are skeptical. To reduce this level of mistrust, mentioning the TU Graz background is essential to gain a leap of trust. Additionally, trust concerns of clients have to be approached already at the beginning of communication with customers.

The correct choice of advertisements is very important and depends on the customer business size, as shown in Table 8-4. This Figure underlines the importance of a target-oriented marketing. It assumed that the target market consists mainly of large companies,

because the operation of industrial-scale bioreactors is very cost expensive. Therefore, advertisements and articles in technical press is highly recommended for a successful marketing.

Table 8-4: Sources of information for buying decisions depending on the company size [77]

Strategy	% Small companies	% Large companies
Trade and technical press	28	60
Salespeople	47	19
Exhibitions	8	12
Mail/Email/content marketing	19	9

In general, advertising is only a form of communication with customers. In the most circumstances, advertising alone does not have a high impact on sale numbers because advertising is only one of many parts (product quality, prices, customer service and competence of sale force) that determines sale levels. Every kind of communication between customer and enterprises needs objectives. The most basic and important communication objectives are education and information, image building, affecting attitudes and loyalty and reminding. The most important advertising objective at the beginning of an enterprise is the creation of awareness, because interest in learning more will usually follow [77]. Specific communication objectives of the enterprise scenario are shown in Table 8-5.

Table 8-5: Fundamental communication objectives of the enterprise

Objective	Detailed description
Education	Awareness creation about modelling bioreactors with novel methods
How	Show: Cost reducing and time saving in comparison to experiments
Image	Create an image of a reliable, trustful and competent company
How	Demonstrate technical leadership based on novel CFD tool
Attitudes	Convince conservative pharmaceutical/ biotechnological companies
How	List advantages of modelling and present success stories
Loyalty	Achieve commitment of customers to gain trust and loyalty
How	Cultivate customer-relationships with intense contact and service

These communication objectives have to be represented in the company's advertising plan. For the enterprise, advertising through different communication media at the beginning have to be cheap and effective. An advertising plan to reach the presented communication objectives is presented in Table 8-6. This concept is also based on the book "marketing plans" [77].

Table 8-6: Advertising plan for the enterprise scenario

Objective	Detailed description
Who	Target audience: R&D heads of biotechnological companies Scepticism about modelling instead of experiments can be expected
What	Objective: Awareness creation for the *Solution Provider* with its tool Response: Interest in the new modelling approach with its advantages
How	Embodiment: High concentration of know-how and expertise Evidence: Long-time partnership with successful pharma company
Where	Exposure: Website/ professional journals (online)/ personal contact Beneficial places: Website/ personal contact
When	Detailed actions for awareness creation should be fixed in spin-off Articles in journals with a relation to the enterprise after spin-off
Results	Expectation: Target revenue of 160,000 € in the first year possible Rating results based on the achieved revenue and number of customers
Budget	10,000 € after spin-off Continuous spending in the starting phase
Schedule	Website creation and advertising in journals directly after spin-off Maintaining website extension and advertising during first three years

The presented advertising plan is developed to reach the marketing objectives presented at the beginning of this section. As already mentioned, advertising is not the only key to success. Customer service, service quality, prices as well as professional behavior also determines the success of a company. Therefore, it is a must that all success determining parameters besides advertising are on point and not neglected.

Summary of main marketing objectives and strategies

In the first year, the enterprise should reach an annual revenue of 160,000 €. After the third year, an annual revenue of about 350,000 € is possible. To reach these planned

revenues, a marketing strategy based on product, price, promotion and place is developed. An appropriate advertising plan is developed in a basic form. It is necessary to know that success parameter like customer service, service quality or prices are of the same importance as advertising.

9 Conclusion and outlook

In the course of this thesis, a CFD tool was extended with biological models and evaluated regarding its market potential. Two non-segregated biological models were implemented and coupled to the fluid field with a Euler-Lagrange approach, utilizing already implemented mass-free pellets that represents the microorganisms. To model a full fermentation, simulations were separated in a start-up phase and a fermentation phase, where biological model equations are multiplied with an acceleration factor.

The first model is a non-structured model representing the fermentation of gluconic acid without the consideration of intracellular activities. The second model, the fermentation of yeast, is based on a structured approach with the consideration of intracellular interactions. The Euler-Lagrange approach activates the biological reaction only in calculation cells with contact to pellets. Recorded lifelines of microorganisms provide more insight into the mixing behavior and spatial concentration distribution in a reactor. Both simulated processes show high accuracy compared to concentration profiles provided by the simple biological models. Comparing the biomass trends, a mean absolute percentage error of 4.0 % is reached for the gluconic acid fermentation and a mean absolute percentage error of 0.5% is reached for the yeast fermentation. The pellet number has significant impact on the results. Thus, slightly more pellets should be used than available fluid calculation cells. A higher number of time steps leads to a slightly smaller error due to a longer mixing time. The biological reactions were carried out with the oxygen transfer rates provided by the simple biological model.

A market analysis was carried out to enable insight into the development and status quo of the biotechnological market. Main focus was on the biopharmaceutical industry. This industry sector is predicted to maintain a strong growth, and in further consequence, an increase of new bioprocesses can also be expected. The trend of single-use bioreactors is considered to have a major impact on the industry. To rate the strengths and capabilities of the tool, other available CFD tools were analyzed in a competitive analysis. Based on the findings it can be said that the in-house developed tool is faster and more optimized

on modelling industrial-scale bioreactors than competitive tools. A SWOT-analysis of the tool was carried out to derive strategies for utilizing the maximum potential in the following business model. For the business model, the most realistic selling concept is defined as *Solution Provider* due to manageable additional code development and best utilization of gathered know-how of the project team. Based on this concept, a business model was derived. Additionally, a market analysis and competitive analysis for a *Solution Provider* was carried out. With the findings, the first three operational years of the enterprise were assumed. A revenue of 160,000 € in the first year and 350,000 € in the third year seems realistic. Costs and state subsidies were also considered in this estimation. To achieve the predicted revenue, a marketing strategy was developed. Recommendations for business communication and advertising were made to give a good starting point for further strategic developments.

9.1 Outlook

The presented approach for modeling whole bioprocesses can reproduce the simple biological models with high accuracy. To improve the simulations' level of detail, fluid viscosity could be coupled with concentration dependent viscosity functions, as applied in [11]. A utilization of the tool's oxygen model for accelerated fermentations could be accomplished by dividing the bioreactor in several zones and couple the number of bubbles with the oxygen concentrations in them. To handle all four types of mechanistic biological models, the implementation of population balance equations for modelling different cell stages has to be considered. However, the implementation of additional non-segregated biological models can be accomplished in an acceptable timeframe which increases the competitiveness of the in-house developed CFD tool.

An estimation if a commercialization of the presented tool has a market potential or not can be made with the accomplished analyzations of the economic part. The predicted development of the biopharmaceutical market and the benefits of CFD simulations for biotechnological companies are supported by literature statements about an increasing importance of CFD in this sector. However, there are still open issues that need to be clarified before a commercialization of the CFD tool is possible. Hurdles are legal

questions, validation issues, code development issues as well as the lack of a customer base. The “Spin-Off Fellowship” by *FFG* provides a good framework, where issues listed above can be solved and further market preparation, development of a detailed business plan and knowledge gathering can be accomplished. A detailed business plan is necessary to reduce the potential risks that go hand in hand with a startup and to convince possible investors. However, based on the findings of this thesis it can be stated that the in-house developed CFD tool is able to compete with other CFD tools in the market. In particular, its specialization on industrial-scale bioreactor modelling makes it unique. In combination with the business model of a *Solution Provider*, a successful establishment in the market can be expected.

10 Appendix

10.1 Table of symbols

Symbol	Unit	Description
C	kg/m³	Mass concentration
x, y, z	m	Cartesian coordinates
k	1/h	General rate constant
k_1	1/h	Rate constant forward reaction
k_{-1}	1/h	Rate constant backward reaction
k_2	1/h	Rate constant enzyme-substrate reaction
t	s	Time
v_m	kg/m³h	Maximum reaction rate
K_M	kg/m³	Michaelis-Menten constant
K_S	kg/m³	Monod saturation constant
µ	1/h	Specific growth rate
r	kg/m³h	Reaction rate
m	kg	Mass
Q	kJ/h	Produced heat of reaction
e	g/g	Key enzyme concentrations
C	g/g	Intracellular carbohydrate storage
g	m/s²	Gravitational acceleration
Subscript		
P		Product
S		Substrate
X		Biomass
O_d		Dissolved oxygen
CO_2		Carbon dioxide
ES		Enzyme-substrate
m		Maximum

Symbols of biological models are explained in Table 10-1 and in Table 10-2.

10.2 Additional equations

Cybernetic variables of the yeast fermentation are represented by u_i and v_i and are shown in Equation 10-1 and 10-2.

$$u_i = \frac{r_i}{\sum_j r_j} \tag{10-1}$$

$$v_i = \frac{r_i}{max_j r_j} \tag{10-2}$$

Carbon dioxide production rate in kg CO_2 and produced heat in kJ are described in Equation 10-3 and 10-4.

$$r_{CO_2} = \frac{dm_{CO_2}}{dt} = \left(\sum_i \frac{\phi_1 * r_i * v_i}{Y_i} \right) * C_X \tag{10-3}$$

$$r_Q = \frac{dQ}{dt} = \frac{dC_G}{dt} * 728{,}42 \tag{10-4}$$

The mean absolute percentage error (MAPE) is described by Equation 10-5.

$$MAPE = \frac{\sum_i \left(\frac{C_{Modelled} - C_{Reference}}{C_{Reference}} \right)^2}{n_i} \tag{10-5}$$

10.3 Simulation parameter

Table 10-1: Model parameter for the gluconic acid biologic model

Parameter	Unit	Description	Value
μ_m	h^{-1}	Maximum specific growth rate	0.688
α	-	Rate parameter	2.92
β	h^{-1}	Reaction rate parameter	0.131
γ	-	Rate constant	2.12
δ	-	Rate constant	0.278
λ	h^{-1}	Rate constant	0.232
φ	h^{-1}	Rate constant	$4.9*10^{-3}$
K_S	g/dm³	Monod substrate saturation constant	130.9
K_O	g/dm³	Monod oxygen limitation constant	$3.6*10^{-4}$
K_La	s-1	Volumetric mass transfer coefficient	0.017
C_d^*	g/dm³	Oxygen saturation concentration	0.0075

Table 10-2: Model parameter for the yeast biologic model

Parameter	Unit	Description	Value
$\mu_{1,m}$	h^{-1}	Maximum specific growth rate glucose fermentation	0.45
$\mu_{2,m}$	h^{-1}	Maximum specific growth rate ethanol oxidation	0.2
$\mu_{3,m}$	h^{-1}	Maximum specific growth rate glucose oxidation	0.2
α	h^{-1}	Specific enzymatic synthesis rate	1
β	h^{-1}	Specific enzymatic degradation rate	0.2
Y_1	g/g	Yield coefficient of glucose fermentation	0.15
Y_2	g/g	Yield coefficient of ethanol oxidation	0.74

Parameter	Unit	Description	Value
Y_3	g/g	Yield coefficient of the glucose oxidation	0.5
K_1	g/l	Michaelis constant of the glucose fermentation	0.1
K_2	g/l	Michaelis constant of the ethanol oxidation	0.02
K_3	g/l	Michaelis constant of the glucose oxidation	0.001
Φ_1	g/g	Stoichiometric parameter	0.41
Φ_2	g/g	Stoichiometric parameter	1.067
Φ_3	g/g	Stoichiometric parameter	2.087
K_{O2}	mg/l	Oxidative pathway saturation constant	0.0001
K_{O3}	mg/l	Oxidative pathway saturation constant	0.0001
γ_1	g/g	Stoichiometric parameter	10
γ_2	g/g	Stoichiometric parameter	10
γ_3	g/g	Stoichiometric parameter	0.1
K_La	s-1	Volumetric mass transfer coefficient	0.097
C_d^*	g/dm³	Oxygen saturation concentration	0.0075
α^*	g/h	Constitutive enzyme synthesis	0.1

Table 10-3: Bioreactor and fluid parameter

Parameter	Unit	Symbol	Value
Reactor diameter	m	-	0.44
Broth weight	kg	-	154.33
Calculation nodes per meter	-	-	179
Liquid density	kg/m³	ρ	1,000
Initial diameter of bubbles	m	-	0.004
Baffle width	m	-	0.045

Parameter	Unit	Symbol	Value
Stirrer speed	min^{-1}	N	190
Shaft diameter	m	-	0.05
First stirrer height	m	-	0.145
Second stirrer height	m	-	0.465
Third stirrer height	m	-	0.76
Stirrer blade outer diameter	m	D	0.147
Stirrer blade number	-	-	6
Position substrate feed (radius, height, angle)	m, m, °	-	0.08, 0.35, 75
Position biomass/ pellets feed (radius, height, angle)	m, m, °	-	0.15, 0.1, 0
Shear rate at which pellets are broken	1/s	-	10,000
Viscosity	Pa·s	-	0.001

10.4 Cost estimation

Table 10-4: The solutions provider's operative result of the first 36 months

		Earnings in €		Receipts in €	
Month	**Fix costs in €**	**Pre-tax**	**After-tax**	**Net**	**Cumulative**
0	0	0	0	30,000	30,000
1	-14,000	12,500	10,000	-4,000	26,000
2	-14,000	11,000	8,800	-5,200	20,800
3	-14,000	12,500	10,000	-4,000	16,800
4	-14,000	11,000	8,800	-5,200	11,600
5	-14,000	11,000	8,800	-5,200	6,400
6	-14,000	12,500	10,000	16,500	22,900
7	-14,000	12,500	10,000	-4,000	18,900
8	-14,000	13,000	10,400	-3,600	15,300
9	-14,000	14,000	11,200	-2,800	12,500
10	-14,000	15,000	12,000	-2,000	10,500
11	-14,000	17,000	13,600	-400	10,100
12	-14,000	18,000	14,400	400	12,500
13	-14,000	19,000	15,200	1,200	11,700
14	-14,000	20,000	16,000	2,000	13,700
15	-14,000	20,000	16,000	2,000	15,700
16	-14,000	20,000	16,000	2,000	17,700
17	-14,000	20,000	16,000	2,000	19,700
18	-14,000	20,000	16,000	2,000	21,700
19	-14,000	20,000	16,000	2,000	23,700
20	-14,000	20,000	16,000	-8,000	15,700

		Earnings in €		Receipts in €	
Month	**Fix costs in €**	**Pre-tax**	**After-tax**	**Net**	**Cumulative**
21	-14,000	20,000	16,000	2,000	17,700
22	-14,000	20,000	16,000	2,000	19,700
23	-15,000	25,000	20,000	5,000	24,700
24	-19,000	26,000	20,800	-14,200	10,500
25	-19,000	26,000	20,800	1,800	12,300
26	-19,000	26,000	20,800	1,800	14,100
27	-19,000	28,000	22,400	3,400	17,500
28	-19,000	30,000	24,000	5,000	22,500
29	-19,000	30,000	24,000	5,000	27,500
30	-19,000	30,000	24,000	5,000	32,500
31	-19,000	30,000	24,000	5,000	27,500
32	-19,000	30,000	24,000	5,000	42,500
33	-19,000	30,000	24,000	5,000	47,500
34	-19,000	30,000	24,000	5,000	52,500
35	-19,000	30,000	24,000	5,000	57,500
36	-19,000	30,000	24,000	-12,000	45,500

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