A Systematic Computer-aided Framework for Development of Pedagogical Process Simulators using Gamification Elements

A fermentation case study

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Abstract

Simulators are proven valuable to enhance intuitive learning based on creating virtual hands-on experiences. However, it is common that the simulators used in engineering education are conceived for simulating rigorous designs and with an analyses aim. Therefore, their primary objective is not educational and it is not taking advantages of novel teaching methods that could engage the student in the learning process. Based on these ideas, user need identification among students of different educational levels in the Department of Chemical and Biochemical Engineering at the Technical University of Denmark (DTU) was carried out; in which an interest in the use of simulators, for the trial of fictional and non-fictional scenarios, and gamification with an educational goal was identified. Moreover, the literature research showed a lack of a simulator with these characteristics and even a methodology that integrated all those elements. Therefore, this thesis presents a systematic methodology implemented in a computer-aided framework for the development of pedagogical simulation tools with the integration of gamification elements. The application of the methodology was demonstrated through a case study.

This computer-aided framework is hierarchical and consists of five sequential steps. First, a need is established to determine the educational gaps, and therefore, the definition of a learning goal. Then, the description of the process including the mathematical and pedagogical description is made. Once the process has been defined, the model for the simulation of the process is constructed and implemented in a computer-aided software tool. It is noteworthy the use of template models to facilitate model creation and reuse. Next, possible elements for a game-based environment through the use of gamification are identified. Finally, the weak points of the simulator are evaluated through a pedagogical verification, which can provide information for the modification or validation of the learning design and/or the integration of game elements, as well as to involve the future users in the design of the tool.

In highlight the application of the proposed methodology, a case study on aerobic growth of *Saccharomyces cerevisiae* on glucose and in a stirred tank batch bioreactor was considered. The validation of the developed process was performed through a learner experience among students in their 2nd year of the bachelor of Sustainable Biotechnology at the Aalborg University (AAU) of Denmark and it was found that 100% of the participating students agree with the usefulness of the developed tool. Also, the students were involved in the design of the platform and as a result, they asked for the addition of more tasks to make the learning process more challenging.

Furthermore, prior to a future learning experience, the case study of the methodology was implemented in a software platform, called FermProc. FermProc is the direct implementation of the proposed methodology and its case study. The implementation was done in Python and its software architecture was designed with the presence of learning hints, interactive questionnaires and the modification of the kinetic model. The first version of FermProc is functional and subject to further improvements pending a second learning experience.

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Nomenclature

Y_{XG}^{Ox}	$\frac{g\ G}{g\ X}$	Yield for the oxidative pathway of glucose to biomass
Y_{XG}^{Red}	$\frac{g\ G}{g\ X}$	Yield for the reductive pathway of glucose to biomass
Y_{XE}	$\frac{g\ X}{g\ E}$	Yield of the pathway of ethanol to biomass
Y_{OG}	$\frac{g\ O}{g\ G}$	Yield of the need of oxygen to glucose
Y_{EG}	$\frac{g\ E}{g\ G}$	Yield of the conversion of glucose in ethanol
Y_{OE}	$\frac{g\ O}{g\ E}$	Yield of the need of oxygen to ethanol
q_G	$\frac{g\ G}{g\ X \cdot h}$	Specific glucose uptake rate
q_O	$\frac{g\ O}{g\ X \cdot h}$	Specific oxygen uptake rate
q_E	$\frac{g\ E}{g\ X \cdot h}$	Specific ethanol uptake rate
K_G	$\frac{g\ G}{l}$	Saturation parameter for glucose uptake
K_O	$\frac{g\ O}{l}$	Saturation parameter for oxygen uptake
K_E	$\frac{g\ E}{l}$	Saturation parameter for ethanol uptake
K_I	$\frac{g}{l}$	Inhibition parameter: free glucose inhibits ethanol uptake

t_{lag}	h	Lag time
$C_{O_2}^*$	$\frac{g\ O}{l}$	Concentration of saturated oxygen
kla	h^{-1}	Mass transfer coefficient for oxygen
C_G	$\frac{g\ G}{l}$	Concentration of glucose
C_{O_2}	$\frac{g\ O}{l}$	Concentration of oxygen
C_E	$\frac{g\ E}{l}$	Concentration of ethanol
C_X	$\frac{g\ X}{l}$	Concentration of biomass
m		Number of components
n		Number of processes
r_m		The rates of each component (m)
Z'_{nxm}		Stoichiometric matrix
ρ_{nx1}	$\frac{g}{l \cdot h}$	Process rate vector

1 Introduction to the project

Education *"is the act or process of imparting or acquitting particular knowledge or skills, as for a profession"* [1]. In engineering disciplines, students are traditionally introduced to theoretical knowledge from lectures and textbooks, after which they may have the opportunity to apply this knowledge in laboratory experiments given the availability of resources. This is a necessary step in order to help the students understand the practical implication of the theoretical knowledge. However, the use of laboratory experiments to impart relevant practical knowledge is becoming more and more challenging, in both industry and academia, due to the staggering rate at which technology has advanced in recent years. Furthermore, a lack of space and resources often restricts the number of students that can be taught in this manner, as well as limits the time they have to fully grasp each concept. As such, new high-throughput educational tools are required to the handle the number of students and adapt to the ever-changing technological landscape. With this in mind, universities and companies are beginning to focus on the development of computer-aided learning tools, such as simulators, for the dissemination of practical knowledge to be used instead of, or in conjunction with, traditional laboratory experiments. These tools should increase the number of students that can be taught simultaneously while minimizing the amount and cost of required resources. Another significant issue faced by the current educational system is to find a way of making complex theoretical knowledge more approachable and to create a long-lasting learning experience that can develop into more in-depth learning. Furthermore, that learning also requires it to be enjoyable. A learning element with the potential to tackle this issue is gamification [2].

This work presents a methodology for the development of pedagogical process simulators with an educational design and using gamification. This thesis combines several areas of knowledge, creating a unique collaboration space between education, biotechnology and chemical engineering, and computer-aided modeling with the aim of advances in the education of future engineers. This project was developed in a collaboration frame between the Process and Systems Engineering Center (PROSYS) in Chemical and Biochemical Engineering Department and the Center for Digital Learning Technology (LearnT), both at the Technical University of Denmark, and the Section of Sustainable Biotechnology at Aalborg University.

The developed systematic methodology was applied on teaching the aerobic growth of Saccharomyces cerevisiae in glucose in a stirred tank batch bioreactor. To do so, the following tasks were performed:

1. Development of a workflow for fermentation in order to provide a theoretical background for the automation of steps without a loss of flexibility.
2. Development of a learning design based on the learning goals, and introducing and testing gamification elements.
3. Development of software architecture and the implementation of the computer-aided modeling framework into a user-friendly software.
4. Validation of the modeling methodology, computer-aided modeling framework, and learning design based on a learning experience or user experience.

Figure 1 shows the timeline in which this project was carried out.

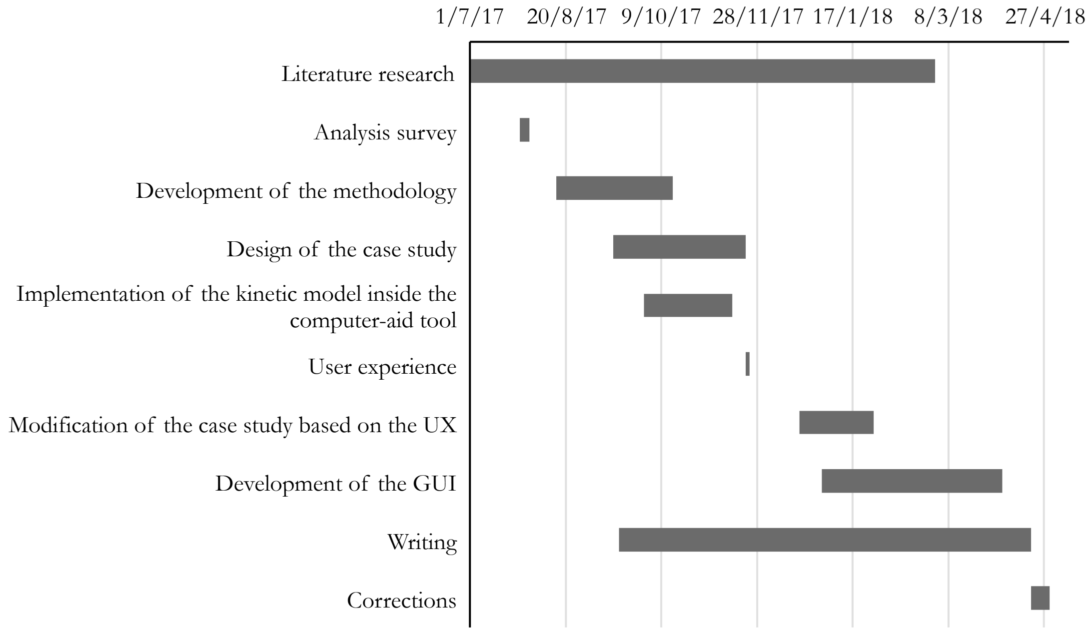


Figure 1. Gantt Chart for the project timetable. It is important to highlight that the task related to writing is so expanded in time due to the submission of a manuscript for the PSE conference, for the SEFI conference and an article, without considering this report. In addition, it was done a course in Learning Technology and Digital Entrepreneurship in DTU Compute between the 13th of September to the 13th of December of 2017, in order to gain more knowledge about the current state and the tendency in e-learning and it is not included inside this Figure.

1.1 Structure of the thesis

This master thesis consists of 10 chapters (including this chapter) and a brief summary of the contents given in each chapter is listed below:

Chapter 2: User Need identification

It is done based on a survey in the Process and Systems Engineering Centre (PROSYS) at the Technical University of Denmark in 2017. In this survey, it was established the student perception of their capability to connect theory and practice, their interest on a simulator for the trial of fictional and non-fictional scenarios, and on the introduction of game elements in their education. Furthermore, in the same survey, 4 beta teachers were asked about their perception of the educational system and the use of simulators.

Chapter 3: Review of existing simulators in engineering education

After the user needed identification, a literature research was performed to elicit on the current-state of-art for existing simulators employed in engineering education, and more specifically their use for the teaching of fermentation. Also, the advantages and disadvantages of using simulators in engineering education are described in this chapter.

Chapter 4: Review of learning in engineering

A thoughtful learning design is a fundamental part of the creation of an educational platform and, in this chapter, it is presented a brief review of learning design, as well as learning theory to explain how learning is taking place. Furthermore, it is more specifically explained the Kolb's experiential learning cycle, as it is the learning theory used further in this project.

Chapter 5: Review of gamification in engineering education

In this chapter, the two main tendencies in the use of games in education are addressed. The advantages and disadvantages of using gamification, as well as the current state of gamification in engineering education are reviewed.

Chapter 6: Computer-aided framework for the integration of learning design and gamification elements

In this chapter, it is introduced the core of this project. A methodology for integrating a thoughtful learning design and gamification elements is presented and implemented through a computer-aided framework. The framework has been created with the use of template-based modeling, and each step of the framework is individually described.

Chapter 7: A case study for the teaching of the aerobic batch growth of S. cerevisiae in glucose

The application of the computer-aided framework was highlighted through a case study for the teaching of the aerobic growth of *Saccharomyces cerevisiae* in glucose and its by-product ethanol in a stirred tank batch bioreactor, with a thoughtful learning design and the introduction of game elements. Furthermore, the computer-aided framework was preliminary validated through a learning (user) experience with students of the 2nd year of the bachelor in Sustainable Biotechnology in Aalborg University of Denmark (AAU) and involving the future users in the design of the tool.

Chapter 8: Assessment of the data obtained in the learning experience

The quantitative and qualitative data obtained in the learning experience are presented and analyzed.

Chapter 9: FermProc: A software platform for the teaching of the case study

FermProc is the outcome software tool from the implementation of the developed computer-aided modeling framework. In fact, a schematic software architecture was created and implemented in Python and PyQt toolkit. Furthermore, it is described an overview of FermProc in its current version. It is important to highlight that FermProc was implemented considering the data obtained from the learning experience and the software is still under development.

Chapter 10: Conclusions and future work

This chapter brings together the more important conclusions of this project as well as a compilation of some possibilities that could not be properly developed due to the lack of time.

Moreover, this project has four appendixes that contain:

Appendix 1: Python code containing the stoichiometric matrix and differential equations for the kinetic model of the case study.

Appendix 2: Experimental settings for the validation of the kinetic model.

Appendix 3: The questionnaires used in the learning experience for the collection of quantitative and qualitative data.

Appendix 4: The brief theory implemented in the FermProc software.

2 User need identification

Initially, a survey regarding the need of simulators for the test of fictional and non-fictional scenarios, and the possibility to use game elements was done at the Process and Systems Engineering Centre (PROSYS) at the Technical University of Denmark in 2017. In addition, the students' perception of the link between theory and practice in the current educational system was asked. In this chapter, the opinions of the 55 students and four teachers asked are analyzed.

2.1 Survey

The proportion of the different educational levels is shown in Table 1.

Table 1. Educational level distribution of a survey done for establishing the need of 55 students (60% male) pre and post-graduated in Chemical and Biochemical Engineering.

Educational level	Percentages (%)
Bachelor of Science	4
Diplomingeniør	27
Master of Science	27
PhD	30
Postdoc	12

Apart for the students, four professors answered the survey. These teachers were selected as beta testers and in September 2018; the department will launch a full-scale survey.

The opinion questions introduced were:

(1) I think the current lecture-exercise format is effective for teaching research-based courses (Q1).
(2) I think the connectivity between theory and practical implementations is clear (Q2).
(3) I can easily convert theory to practical implementations (Q3).
(4) I believe that entering a simulator in which I could play around with the parameters of benchmarked models would help me learn (Q4).
(5) Game-based learning is likely to help motivate my desire to learn. Figure 2 and Figure 3 show the opinion of the participant without considering and considering the educational level respectively (Q5).

This survey looked to cover topics about the feeling of the students for their preparation to the transition from the academy to the industry (Q1, Q2, Q3), and the desired for the test of "what if" (Q4) scenarios or the presence of game elements (Q5). All these topics have a great importance during education.

2.2 Results

Figure 2 shows the cumulative opinion of the students disregarding their educational level, Meanwhile, in Figure 3 the different educational levels are considered for each of the questions in the survey. In Figures 3a), 3b) and 3c) the participation of the teacher is presented towards the other educational levels, while in Figure 3f) the teacher´s opinions on three specific questions is collected.

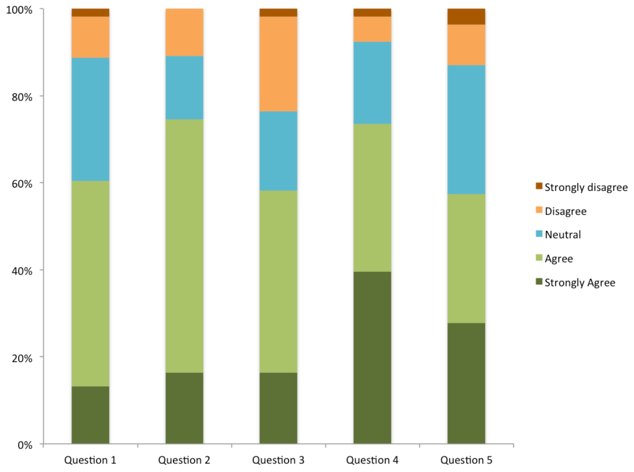


Figure 2. Cumulative graph summarizing the response to the survey of the participating students.

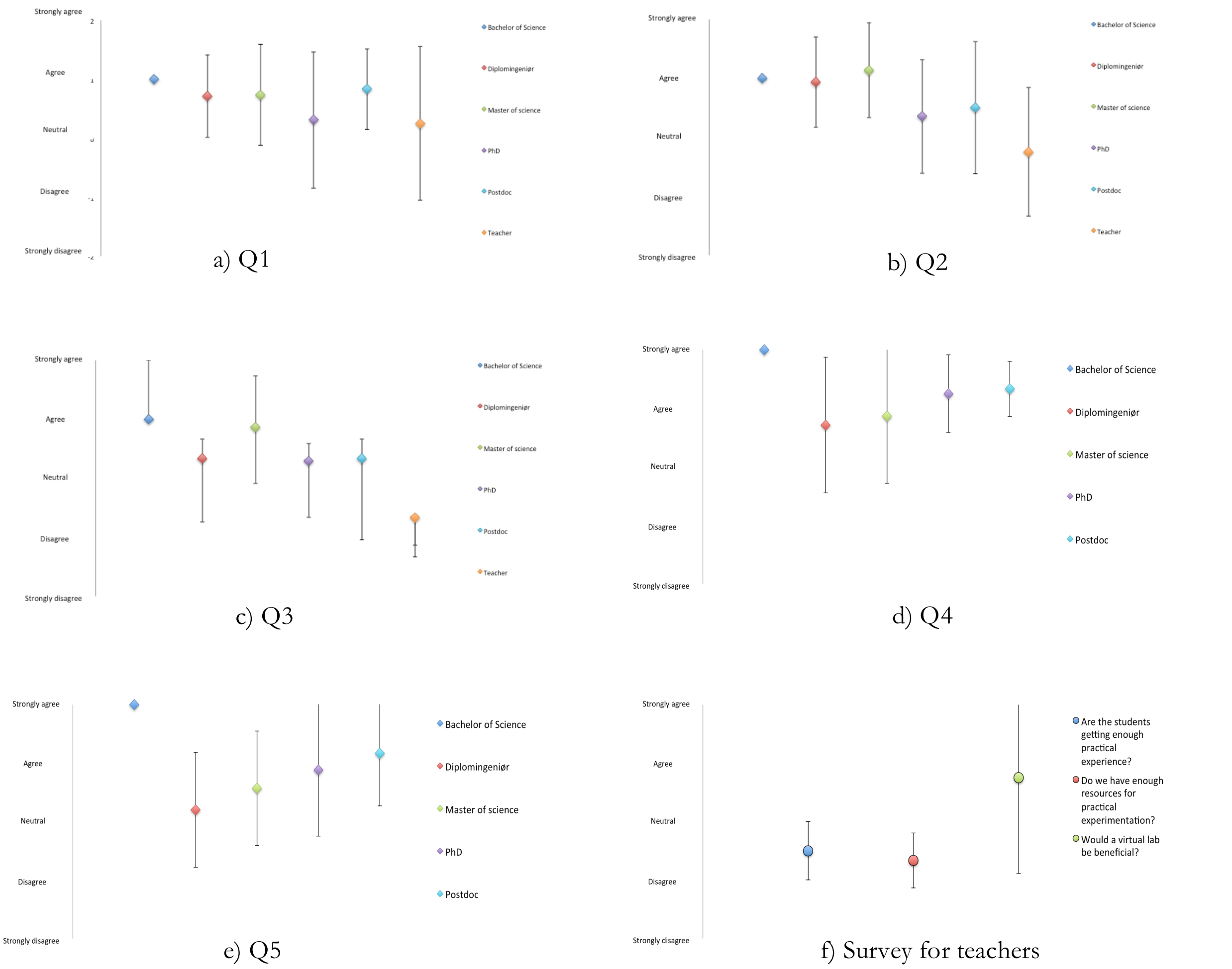


Figure 3. Data collected in a survey to established the need of a tool that use gamification in the explanation of complex process. This data is presented with its standard deviation to give an idea of how spread the opinions are.

2.3 Conclusion

As it can be seen in the cumulative graph of Figure 2, 60% of the students agreed about the effectiveness of the current systems of lecture-exercises (Q1) and 74% believed that the connection between theory and practice is clear (Q2), while 57% agreed that they could easily convert theory to practical implementations (Q3). When they were asked about the use of simulators, 73% of the students believed that a simulator in which they could play around with the parameters of benchmarked models would help them learn (Q4). And 57% of the participants responded positively when they were asked about the use of game-based learning to help motivating of the learning experience (Q5). Furthermore, it can be seen that percentage of students that strongly agreed about the use of gamification (30%) is the half of the students in favor (60%), while in the use of simulators 40% of the participants strongly agreed from 77% of the students were in favor of using simulators. This shows a strong interest from part of the students, and from Figure 3d) and 3e) it can be seen that the participants who showed a stronger interest in the use of simulators and gamification were bachelor students, and after a decrease in the interest from the next educational level, the interest increased with the educational level. It is noteworthy that the low percentage of participants from the bachelor level (4%) makes this conclusion preliminary.

Generally, Figure 3 doesn't show an overall tendency and it is difficult to arrive at any clear conclusion because the high standard deviation presented in all the educational level. However with the increase of the education level, the satisfaction with the current way of teaching decreased in Figures 3 a), b) and c), related to the connection between practice and theoretical knowledge. For example, in Figure 3 c), it decreased from a 100% of the bachelor of science student participants to a 41% of agreement of the PhD students in the ability for the conversion of theory in practical implementation. In contrast with the students, the teachers showed a higher disagreement on the capacity of their students to see the connection or to convert theory to practical implementations and 100% of the teachers asked disagreed about the ability of the students to convert theory in practical implementation. Furthermore, in Figure 3f), 50% of the teacher participants disagreed about the effectiveness of the current lecture-exercise format for teaching research-based courses, while the 50% strongly agreed in the beneficial effect that a virtual laboratory could have for the students. On the other hand, the teachers with more experience and a global image of the education system agreed on the benefits of the use of a virtual laboratories (75%) and the lack of resources for practical experimentation (100%).

To sum up, the survey showed the students interest for a simulator that allows the test of fictional and non fictional scenarios with a practical approach; meanwhile the participants were also interested in the use of gamification, especially to the extreme levels of the educational levels asked. Moreover, teachers generally agreed of the benefits of virtual laboratories and therefore, it can be concluded that there is interest from both teachers and students for simulators with game elements and a playful model.

3 Review of existing simulators in engineering education

The literature review provided in this chapter is an introduction to the background and current use of simulators in engineering education as well as some examples (Chapter 3.1). Furthermore, it is compiled the advantages and disadvantages of its use as a learning tool and future perspectives about its development (Chapter 3.2). Finally, it is briefly summarized some of the existing simulators on the teaching of fermentation (Chapter 3.3).

3.1 Background

As it was mentioned in Chapter 1, the performance of physical laboratory exercises in education has incremented its difficulty due to the need and maintenance of equipment, space, and staff. Therefore, alternatives or complements, such as simulators, have been developed for tackle this safety and economic issues and they have been successfully applied in academia and industry.

Besides, three types of laboratories: development, research, and educational, are used in engineering [3] and these three types can be analogues to today's simulators and virtual environments. They have several shared characteristics, but their differences are fundamental. The development laboratory is used to answer specific question to design and/or develop a process. While a development laboratory has a specific question to answer of immediate importance, research laboratories are used to seek broader knowledge. Students, on the other hand, require for an instructional laboratory to learn *something* practicing engineers are assumed to already know. That *something* needs to be defined by carefully designed learning objectives. And although an educational simulator should be design with an educational aim, the commercial simulators, which are mostly used in engineering education, are mostly designed with a development approach.

Even so, it is commonly accepted that simulators are not prepared to be a stand-alone substitute for physical laboratory exercises, educational simulators have been already used [4]:

- For a pre-lab experience. It can prepare students about what they will encounter in an actual experiment. It can be, for example, with the use of virtual reality such as in LABSTER software [5].
- For experimental studies of systems that are too large, too expensive, or too dangerous for physical measurements [6]–[9].

Simulators have the capability to helping students gain more theoretical knowledge and conceptual understanding [3], [10] due to the manipulation of the models implemented allowing an intuitive learning based on action and offering the student a feeling of control and enhancing explorations. However, the capability of educational simulators depends on the authenticity, constraints, and capabilities of its design as a learning tool and the model implemented [11]. Then, during the last years, more realistic simulations have been developed with a collective effort between academy and industry.

3.1.1 Brief overview of some existing simulators in engineering education.

The use of simulators as learning tools in higher education started in the 1970s and many areas such as wastewater treatment [12], [13], robotic [14], electronic circuit [15], control [16], etc. have so far benefited from its use. Some examples of simulators currently use in education are listed in Table 2.

Table 2. Overview of some of the educational simulators available in different areas.

Software	Area	Description	Reference
CyclePlad	Chemistry-Thermodynamics	Virtual Remote Laboratory (VRL) to design and analyze thermodynamic cycles. Also, the software is able to evaluate the student design and propose improvement to such design.	[7]
-	Chemistry	A web-based interactive virtual laboratory system for unit operation and process systems, such as distillation, in different programming languages. In the report, a positive effect is also established in the education of the students who used the software.	[17]
-	Control	A web-based control laboratory for experimentation on a nonlinear multiple-input-multiple-output system: the three-tank plant. This software is written in an open-source.	[16]
GeneSPIDER	Biologic	Biological software for modeling cellular networks. It was implemented in Java.	[18]
KTechLab	Electronic circuit	An open source integrated to design the environment for electronic and PIC microcontroller (family of microcontrollers) circuit design and simulation. It was implemented in C++	[15]
RecurDyn	Mechanical engineering	A computer-aided engineering software system, focused on MultiBody Dynamics, while also offering multiphasic solutions.	[19]
REAL	Robotics	A virtual laboratory for mobile robot experiments. The lab was implemented in Java.	[14]
ICAS	Chemical engineering	Software for the design, validation and analysis of process monitoring and analysis of the manufactured of chemical products.	[20]
WEST	Wastewater treatment	A modeling software system able to simulate physical, biological or chemical processes in wastewater treatment plants, sewer system and rivers.	[12]
SIMBA	Wastewater treatment and biogas	A simulation platform with libraries for dynamic modeling and simulation of wastewater treatment plants, collection systems, and rivers.	[13]

Moreover, some institutions have developed their own operating virtual laboratories for several operational areas interconnected like in the case of the open-source iLab Project of the MIT [21], the Online Widener

Laboratories [22], or the Collaborative Laboratories for Europe [23]. However, it is important to highlight that this kind of initiatives requires continuous server maintenance.

From the simulators gathered in Table 2, WEST and SIMBA# are benchmark simulators. A benchmark simulator is designed to mimic a particular process and therefore offers more detailed information in regards to how the original system actually works. However, this type of software requires previous knowledge of the system and also presents a loss of freedom in the definition of the process. Yet, although they are mostly for development, benchmark simulators can be useful for learners as they provide more information, such as the "normal" values of the parameters of a complete process or the assumptions that need to be made. However, their lack of a learning design decreases its educational power.

3.2 Advantages and disadvantages of the use of simulators in education

Further than the advantages briefly mentioned in the previous section, the main benefits of a laboratory simulator are [4]:

- *Availability*. A simulator can be used anywhere and anytime if a suitable electronic device is available, in contrast with an actual laboratory that needs to avoid the overlapping in the use of equipment and considers working hours.
- *Accessibility*. It doesn't require being physically present in the lab, allowing the experience to disabled people or people without the possibility to access an actual laboratory.
- *Observability*. Simulators can show modified or simplify models to make phenomena more visual like in subjects such as thermodynamics or electric phenomena.
- *Immediacy*. It allows performing a wide range of experiments with immediate feedback and easily repeats the experiment.
- *Safety*. As it has been previously mentioned, simulators allow students not to be in direct contact with hazardous substances and dangerous processes.
- *Reduction of cost*. In comparison to an actual laboratory, it doesn't require space, equipment, etc. although it requires an high initial investment.

Even with these advantages, it is important to highlight that simulators commonly have several issues in its use and design, and therefore physical laboratory experiences are required for a practical education. One of the main disadvantages for the possibility to use simulators instead of physical laboratories is the impossibility of the simulator to provide all the competencies obtained in a physical laboratory. Likewise, physical laboratories provide students with knowledge-based sensory awareness, acquiring psychomotor skills, teamwork experience and ethics in the laboratory [10]. Therefore, new technologies have been applied to tackle those problems and for example, a virtual remote laboratory with leap motion control, that supports hand and finger motions as input, allows the user to move the instruments of the chemistry virtual laboratory (CHEMOTION) in order to enhance the psychomotor experience [24]. Furthermore, a simulator can produce a feeling of isolation for the student, and the loss of real "hands-on" lab experience and real data, which can make students reckless and without actual knowledge of how a lab works. Also, limited pre-design inputs and outputs can decrease the creativity of the student as the application of realistic and unrealistic models in simulators are essential to understanding how the process reacts. To tackle those problems, periodic self-evaluation and the use of communication technology such as a chat supporting collaborative learning, have been proven as an effective way to decrease the isolation of the students [4].

The other important drawback in the implementation of virtual laboratories as a learning tool is due to its default design as development laboratories. Commonly, commercial development virtual laboratories work as black boxes so their mathematical models are not displayed and it is not possible to recognize the assumptions made [25], [26]. Furthermore, as simulators are created as a closed system, their models cannot be freely modified without a high knowledge of the programming language and the model, and consequently, the simulator loses the opportunity to evolve at the same time that as the student.

3.3 Simulators in fermentation

One of the disciplines that benefit from virtual laboratories is biochemical engineering. Biochemical engineering is based on full-scale industrial processes, and providing a hands-on experience for the student can be a challenge considering safety and cost. In the case of the fermentation process, bioreactors in a production facility have a production volumes up to 2500 m^3 [27] and therefore, a physical student laboratory of this production volume is not feasible. Also, the mathematical model behind the description of a fermentation process is complicated and its modeling has several complexity layers. This is because the model has as many variables as properties of the process are considered and therefore the use of an educational simulator for the description of the process can be highly useful. Furthermore, they are a lot of software with and without a pedagogical purpose that are able to simulate fermentation processes, some of them summarized in Table 3.

Each of the software in Table 3, with the exception of AQUASIM and BiotechLAB, works as a black box and therefore it is not possible to visualize the kinetic model. Of those two "open" systems, AQUASIM is the most popular. This software is very flexible in model definitions and is mainly used for the comparison of the model with a data set. However, AQUASIM is a development simulator that lacks from a learning design. Moreover, in this software, it is necessary the implementation of the whole process, with the correct classification of the variables and the process equations, and therefore requires from an expert user with a high level of knowledge of kinetic model.

Another example of educational software but with a different design, is BerryMaker, a game-based simulator. BerryMarker allows the change of the conditions of the fermentation, aiming to maximize the production [28]. Its educational value is based on learning by failure and the use of the players' instinct and therefore, this app does not explain the reason behind the results and lacks from theoretical hints.

Table 3. Overview of some of the most popular fermenter simulators.

Software	Description	Disadvantages	Literature
Labster	Fully interactive advanced lab simulations	The models implemented cannot be visualized and it has a limited pedagogical range	[5]
SmiSci Pro//II	Software for the optimization of the plant performance implemented based on the calculation of rigorous heat and material balances. It is a development simulator.	The software works, as a black box with a predefined set of kinetic expressions and its educational value is limited to the overall process developed.	[29]
Aspen Hysys v 7.3	It is a dynamic process (of development) simulation software.	The software doesn't have implemented any bioconversion but only 5 possible kinetic expressions, limiting the accuracy of the model, as well as it educational value.	[30]
SuperPro Designer	Software for the modeling, evaluation, and optimization of integrated processes. It is a development simulator.	The fermentation unit has predefined models and cannot be modified and therefore, it has limitations for the simulation on the effects of fermentation.	[31]
PLVPQ	A web- server simulator for chemical and biological processes.	Although it can have different possible configurations for the fermenter, it doesn't allow the display of its model or its modification.	[32]
AQUASIM	Software for simulation and data analysis of aquatic systems	It is the most flexible software concerning model definition, as it has to be specified by the users, and therefore the user requires previous knowledge of kinetic models.	[33]
BiotechLAB	A web-server software for the simulation of proposed exercises, and therefore with a clear educational value, and implemented as an open-source.	Although all the models can be visualized, and exercises are implemented as based on learning, the organization of the web page leads to confusion.	[34]

3.4 Conclusion

Simulators are well-established learning tools that can help students gain more theoretical knowledge and conceptual understanding. It is commonly accepted that simulators cannot substitute physical laboratories at the same level as physical laboratories are unable to substitute the exploration and portability capacity of the simulators. The use of simulators as an educational complementary tool greatly depends on a thoughtful design of the learning experience. Even so in engineering education, the existing simulators are commonly for development and consequently, they lack the required learning design. Moreover, the literature research shows a lack of fermentation simulators that allow the visualization and modification of kinetic models combined with a thoughtful learning design.

4 Review of Learning in engineering

As it has been previously mentioned in Chapter 3, simulators used as learning tools sometimes lack of a thoughtful learning design and an educational aimed implementation, such as with the display of the models. In order to provide an optimal framework for the learning process, in this chapter it is done a literature review about the concepts of learning design (Chapter 4.1) and learning theory (Chapter 4.2), as well as described a current learning tendency for a technical high education.

4.1 Learning Design

Learning design can be considered as a framework that supports the learner in the educational experience. Likewise, the learning design describes the teaching and learning process, along with the conditions, learning objective, target group, and a specific context or knowledge domain [35]. Even with the arrival of new technologies that has come with the evolution of the learning process, one thing has not changed. New technologies have not modified what it takes to learn [36]. This statement allows turning to the traditional learning theories to ensure that the learning process exploits and challenges technology.

In this section, it is characterized three different pedagogical principles (instructionism, constructionism, and collaborative learning) that focus on different elements of the learning process with a special interest in the teaching of engineering.

4.1.1 Instructionism

Instructionism refers to an educational practice that is teacher-focused, skilled based, non-interactive, and highly prescribed [37]. Instructional theories prioritized the presentation of the concept by the teacher. It has a goal, which the learner attempts to achieve, and then extrinsic feedback in terms of right/wrong, hints, new material and/or different tasks. Therefore, learner hasn't any opportunity to reflect on the relationship between the goal, their action, and its effects, due to the lack of intrinsic feedback or interaction with other learners. This can be seen in Figure 4.

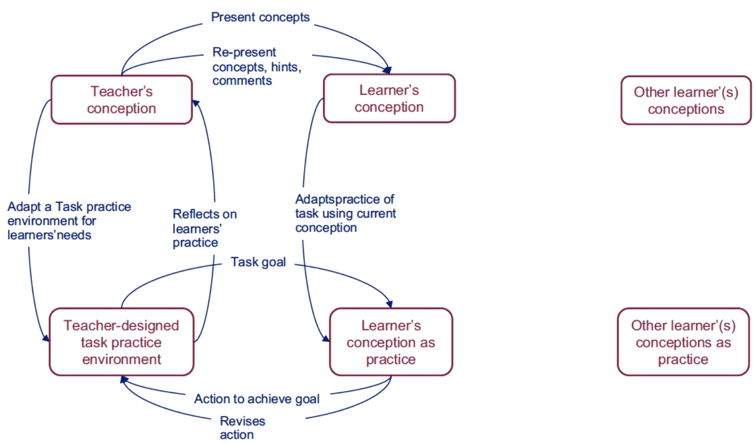


Figure 4. I Instructionism scheme from Laurillard (2008) [36].

4.1.2 Constructionism

Constructionism or constructivism refers to a educational practice that is student-focused, meaning-based, interactive and responsive to the student interest [37]. In this case, the learner develops their conceptual understanding through repeated attempts to achieve the goal and reflecting on the internal relation between the goal, concepts, actions, and feedback. This process enables to adjust the current conception of the learner. Therefore, the focus in this learning design theory is in the intrinsic feedback, from which the learner reflects and promotes an internal deliberation and learning. This can be seen in Figure 5.

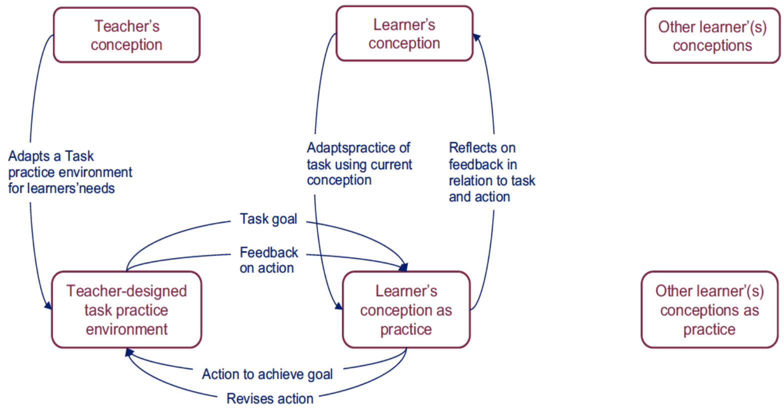


Figure 5. Constructionism scheme from Laurillard (2008) [36].

4.1.3 Collaborative learning

Collaborative learning is the combination of the intrinsic feedback of constructivism and social learning. Here, learners have the chance to share and discuss the decisions that they take and the results. This collaborative environment provides the learner the possibility to build its knowledge from the outputs of its peers and its own results, and encourages sharing his/her reflections. This can be seen in Figure 6.

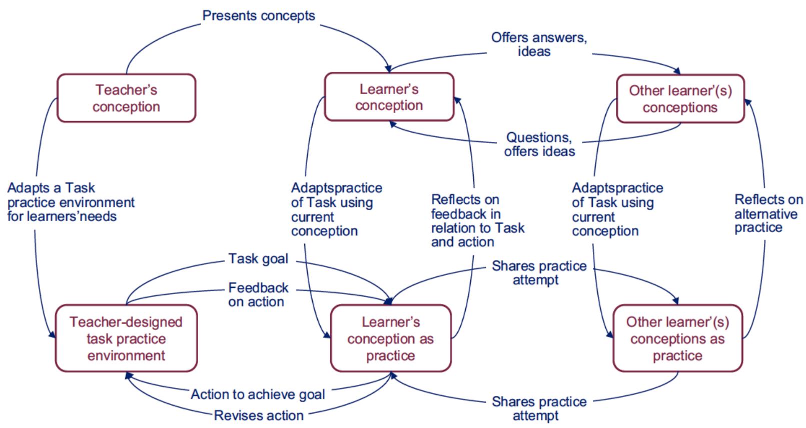


Figure 6. Collaborative learning scheme from Laurillard (2008) [36].

On the other hand, collaborative learning requires of small classes or groups that are actively collaborating and are mentored by the "teacher" or teacher's conception. Therefore, although collaborative learning presents advantages such as the possibility of discussion between learners and the generation of new ideas and interpretations, it is limited by the modern educational problems such as the increased number of students or limited budget. However, those issues can be avoided in e-learning with the creation of an online teaching that supports the communication and collaboration among users [38].

It is important to highlight that in Figures 4, 5 and 6, "the teacher's conception" is the idea in charge of present the concepts and the learning goals. Therefore, it doesn't require to be a teacher, but it can also be a computer platform, etc.

In the specific case of engineering education, constructionism is a more suitable learning design than instructionism due to the practical approach of the field with a focus to develop an analytical thinking and a problem-solving goal. On the other hand, the possible use of collaborative learning will be further explained and discussed in this thesis without its implementation, as the limited time of the project didn't allow it to go farther than the proposal to share opinions among peers.

4.2 Learning Theory

While learning design describes the complete process of learning, learning theories are the conceptual framework describing how learning is absorbed, processed, and retained [39]. The existence of several learning theories and its importance is due to the complexity of the learning. Learning, in a broad sense, involves biologically and societally elements, which follow a different set of logic, and work together in a complex interaction [40]. This section is mainly based on the previous work of K. Illeris (2013) [40], who based the process of learning on three dimensions. These dimensions are the cognitive, the emotional, and the environment; and which interaction can be seen in Figure 7.

The cognitive and emotional dimensions are always initiated by impulses from the interaction processes and are integrated into the internal process of acquisition and elaboration of the individual. Furthermore, the cognitive dimension is related to the learning content, building the understanding and the ability of the learner. And whereas the emotional dimension encompasses mental energy, feelings, and motivations; the environment dimension sets the basic conditions for the learning.

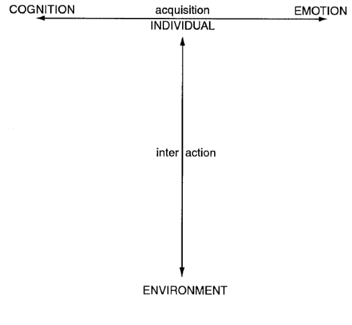


Figure 7. Scheme of the fundamental dimensions and processes of learning from Illeris (2003) [40].

Based on the scheme seen in Figure 7, learning is a constructivist or a collaborative-learning concept in its nature, as the learner adapts its knowledge based on a reflection generated by its own experience [41]. It is important to highlight that constructionism is applied to both; to how people learn and to the nature of knowledge [41].

Furthermore, learning has to be structured before it can be retained. However, the structuring of learning could happen in different ways, and on this basis it can be distinguished four different levels of learning for the design of the learning process.

- *Cumulative.* It is characterized by being an isolated formation; something new that is not a part of anything else. It is normally at the beginning of life, for example, walking. It is also related to the learning of something of personal importance with no previous link, like a pin-code.
- *Assimilative.* It is the most common form of learning. It is learning by addition, in which new knowledge is linked as an addition to a mental model already established.
- *Accommodative.* This learning needs for the break up of an already existing mental model and its transformation so new information can be linked.
- *Transformative.* This level of learning implies what could be termed personality changes and is characterized by a simultaneous restructuring in the cognitive, emotional and the social dimensions and usually occurs due to a crisis-like situation.

Another important aspect of learning is when it is not able to happen. This can be due to mental resistance or mental defense. Mental defense is more importantly for this project. Mental defense is an automatic mechanism, activated when new information does not correspond with pre-understanding so it is rejected or distorted. Therefore, it is important to provide the learner trustful sources and, in order to establish if the learning has taken place, it is necessary to integrate an evaluation system.

Overall, many learning theories about how learning is taking place had been developed and implemented. In this report, only the Kolb's experiential learning cycle is explained, as it will be farther used in Chapter 7.

4.2.1 Kolb's Experiential Learning Cycle

The Kolb's experiential learning cycle is a learning theory based on a constructivist view with a cyclical model of learning that can be seen in Figure 8 [42]. This theory has been implemented successfully for the education of engineering for example in the Engineering Design department at the Technical University of Berlin or in Introduction to Robotics at the University of Colorado at Colorado Springs (UCCS), as well as in the Chemical Engineering Department at Loughborough University, United Kingdom [43].

As it is shown in Figure 8, Kolb proposed an effective learning that progresses through a cycle of four stages. Initially (1) a new experience or situation or the reinterpretation of an existing experience is confronted, and is followed by (2) an observation and reflection on that experience which leads to (3) the formation of abstract concepts and generalizations or conclusion which are then (4) used to test the hypothesis in future situations, resulting in new experiences. Therefore, the Kolb's experiential learning theory involves the following phases: stimulation, reflection, abstraction, and experimentation. These steps, related to the solution of a technical problem and to an analytical thinking, are necessary abilities for engineers.

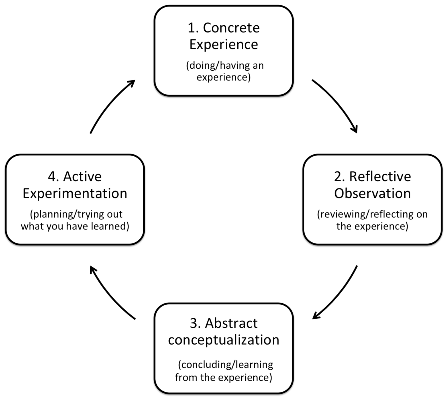


Figure 8. Kolb's Experiential Learning Cycle based on the previous work of Kolb [42].

4.3 Conclusion

Based on the information collected, it is possible to describe the learning process wanted for a process simulator. It is characterized as assimilative and a predominant cognitive learning, although it considers and implements emotional and environment elements. However, the decision of the learning design implemented was more complicated as the restrictive time of the project withdraw the possibility to implement a collaborative environment. Therefore, based on the literature research it is used a constructive learning design (Figure 5) with the integration of Kolb's experiential cycle (Figure 8) as learning theory in the creation of fictional and non-fictional scenarios and with the future perspective of creating a collaborative environment between peers in the future.

5 Review of Gamification in engineering education

As it was explained in the previous chapter, learning requires an emotional dimension. This emotional engagement of the student is one of the challenge of the current education system as, in a lot of cases, the learning was designed without considering the new habits and interest of the students [44]. In this context, the use of games or the introduction of game elements inside a pedagogical platform may create a new learning process that corresponds better with the new needs [45].

Therefore, in this chapter is established the difference between game-based learning and gamification, the advantages and disadvantages of gamification and a brief overview of the current state-of-art of gamification in engineering education.

5.1 Game-based learning versus Gamification

Game-Based Learning is similar to Problem Based Learning, a well-establish instructional method in the tertiary education, wherein specific scenarios are placed within a play framework [46]. In addition to the Game-based learning, another concept called "Gamification" appeared with a pedagogical perspective. Gamification is the use of game elements in non-game contexts [47].

The borders between game-based learning and gamification can be confused, as both of them are applying the same principle, the use of games, to enhance the learning process. However, while gamification uses game mechanics to change the learning experience, the game-based learning incorporates online games into the learning process to teach or develops a particular skill [48].

In a previous work done by Jayasinghe and Dharmaratne (2013) [49] with university students of Computer Science, it was tested the two scenarios of the used of gamification techniques and game-based learning in order to know the preference of the students. Based on a learning experience, it was seen that the learners choose the use of gamification and, moreover they were capable of understanding the underlying theories easily with the gamified component. Furthermore, another application of gamification in tertiary education, for mechanical engineers, has been proven as an excellent tool for engage the student and increase incidental learning [46].

5.2 Advantages and disadvantages of gamification

The benefits of learning through games are numerous and games are often closer to simulating real-life experiences that more traditional educational media, and allowing the students to immerse themselves in a realistic simulated setting without the fear of real-life consequences [46]. Therefore, the use gamification can help in the teaching of fictional and non-fictional scenarios in fields that require an intensive practice experience before acquiring technical competencies needed in real life, such as engineering.

On the other hand, new technology sometimes presents a number of issues related to social implementation and use, and gamification has been criticized because [50]:

- It is not systemic as it is merely added game elements.
- It is reward-oriented, instead of intrinsic motivated. This is due to the presence of two different goals; the learning goal and the game goal, and whereas the learning goal is the knowledge and intellectual abilities that the student learns through the process, the game goal is the actual goal the student/user is striving for in the environment [51].
- It is not user-centric, as the developer is in charge of establishing the goal and not the student.
- It is pattern-bound, and therefore the feedback interface is commonly limited to a small set of options.

However, those issues can be tackled by different strategies. Firstly, it is integrated a framework for gamification developed by Weitze (2012) [2] to avoid the lack of systematization. Moreover, due to the choice of a constructivist learning, the platform needs from an intrinsic feedback in charge of motivate the students to reflect about its own learning and therefore, it is part of the design requirements to implement a variable feedback. Furthermore, the platform is developed with a close collaboration between the developer and the future users as an integrated system for the learning and gamification design.

5.3 Current state-of-art of gamification

In the industry, gamification-based training has promised to maximize profits and employee productivity [52]. For example, mobile services such as Nike+ has created the bridge that connects gamification with marketing, while Pep Boys (an automobile engineering company) reduced in a 45% its safety incidents with the introduction of gamification into their training program [53]. While gamification has gained its ground in business, marketing, corporate management and wellness initiatives, its application in high technical education is still an emerging trend [54].

The possible use of gamification in engineering education has been more extensively described that the actual implementation guidelines of the gamified designs [55] or on the effectiveness of incorporating game elements in an engineering learning environment [56]. Only a few references have been found of empirical implementation of gamification such as for mechanical engineering students [46], [57] or computer science students [49] and commonly they are missing of a deeper explanation of how game elements are incorporated into the learning design.

5.4 Conclusion

The literature research has found a lack for the design and implementation guidelines of game elements in simulators for engineering education, and therefore, the focus of this project is the creation of a methodology for the integration of game elements inside educational processes simulators. Indeed, in this chapter, it has been described two different learning approach of the use of "game" in education and based on literature, it was chosen the use of gamification to engage the students in the learning process. However, in order to avoid the common issues found in the implementation of gamification, it is used a previously developed framework and a close collaboration between the developer and the future users.

6 Computer-aided framework with the integration of learning design and gamification elements

In Chapter 2, it has been established the user's interest on educational software that enables to try fictional and non-fictional scenarios combining game elements. Additionally, a literature research (Chapter 3) has shown a lack of laboratories simulators with those characteristics, and moreover, the introduction of game elements in educational process simulators lacks for an integrated design and implementation guidelines (in Chapter 6). Therefore, it is develop a systematic methodology that supported the development of model simulation with an experiential and gamified approach.

A methodology allows the systematization of the creation of structures, while a computer-aided framework supports the architecture through computer-aided methods and tools that can be implemented and used. In this chapter, a computer-aided framework that integrates the use of a pedagogical approach, of gamification, and the construction of the simulation of mathematical models is developed. The resulting framework, including methods and information flow, is presented in Figure 9, and consists of five hierarchal steps, four of them for model development and one for model application (Step 1-4) and validation (Step 5). The framework is also generic, and therefore each step can be applied independently based on the availability of input information; and iterative as the model development process is evaluated and refined until the optimal process is found.

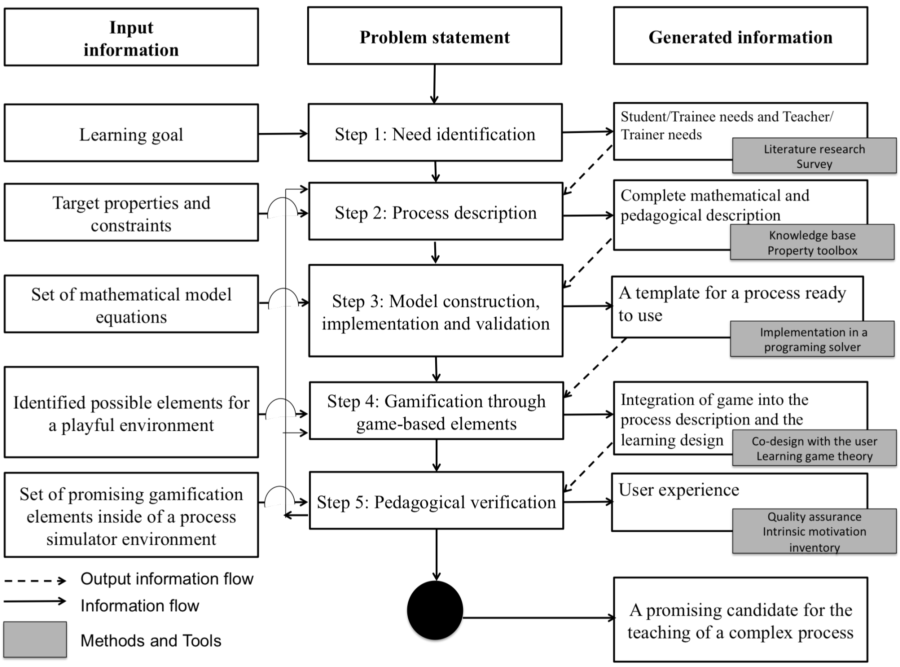


Figure 9. Framework for the development of a pedagogical simulation tool.

In the forthcoming sub-sections each step of the framework is deeply explained, and in Chapter 7, it is applied to a specific case study focused on the teaching of an aerobic batch fermentation process with *Saccharomyces cerevisiae* to students with only elemental pre-knowledge of the operation of a fermentation process.

6.1 Step 1: Need identification

Objective: Establish the need that has to be fulfilled through a pedagogical approach. Therefore a learning goal, that students should reach, is defined.

Note: The learning goals that are defined cover the knowledge, skills and competencies that the students should have acquired when the learning process is completed.

Step 1.1: The knowledge and intellectual abilities that students are expected to acquire need to be defined. For example in the case of a biochemical engineering process, this need may be to provide competencies associated with any process and unit operation design.

Step 1.2: The objectives to be fulfilled as a result of satisfying the identified need may be qualitative in terms of a learning design and quantitative through a set of properties and constraints. Therefore, the learning goal will then be used as an input that supports decision-making to describe a given process.

Tools: This need established is usually set up by either user-need (often quantified through a survey) and/or a comprehensive literature review.

6.2 Step 2: Process description

Objective: The development of the description of the process as well as the learning design to fulfil the learning goal. Furthermore, the learning goal provides of a series of target properties and constraints.

Step 2.1: The process system information is collected in a series of gradual and detailed steps based on rhe previous work of Heitzig *et al* (2010) [26]. It can be done as follows:

1. Provide a functional description or a sketch of a system to be modelled. This includes balances, volumes, phases, components, of the system and their functions.
2. Provide system conditions. General conditions for the actual modelling problem, for example, temperature, pressure, etc.
3. List phenomena in the system that might be of importance.
4. Collect information on modelling of the system/problem at hand. This includes information on how a system can be modelled or similar systems have been modelled.
5. Set up a list of potential assumptions that can be used to simplify the model of a system.
6. Collect preliminary system/ process/ reactor data. For example, experimental data, initial values guesses for parameters and variables.
7. Select model scenarios of interest.

<u>Note</u>: Therefore, the process system requires information related to the pure component properties, reaction rate constants, transfer coefficients and other relevant process parameters that are stored in a property toolbox, supported by information collected from the literature, databases, model libraries, databases, expert knowledge, or experience and experimental data.

<u>Step 2.2</u>: On the other hand, a didactic model frame is used for the development of the learning design. This frame describes six important areas that should be defined for the planning and carrying out of the teaching process, as proposed by Hiim and Hipee (1997) [58] and modified and used by Weitze (2012) [2]:

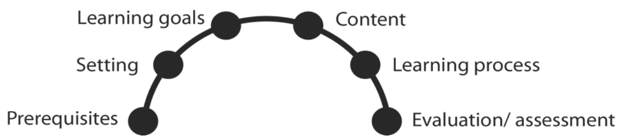


Figure 10. Frame for the Design for learning by Weitze (2016) [2].

Therefore, based on Figure 10, the process description for the learning design needs to tackle all the points in the frame and define the prerequisites of learning, related to the target group, previous knowledge should be considered, motivation to complete the task, etc. Also, the frame in which learning is taking place (setting) needs to be established or designed.

The learning goals involve the knowledge, skills, and competencies the students should have acquired by the end of the course. The content describes what information the teachers present for the students in order for them to reach these goals. Moreover, the learning process involves the activities the teacher plans for the students in order for them to learn and thereby reach the learning goals. The learning goals can be broken down into sub-goals addressing objectives for the students at various levels of cognitive complexity [59]. The teacher then has to find suitable content for the students to engage with, in order for them to reach these different sub-goals. Based on that content or teaching material, the students have to learn and consequently go through a learning process. Here, the teacher often bases his learning design on a specific pedagogical belief. For example, such a belief can be that students learn best through collaborative learning processes, i.e. by working together in the planned learning activities. Another example is when a teacher applies Kolb's experiential learning cycle to the planning of activities in order to support specific parts of the learning processes of the students. After the students have been through various learning processes, the teacher needs to assess and evaluate if the students have reached the intended learning goals.

<u>Note:</u> It is noteworthy that these sub-steps are also generic and either (or both) a mathematical model and/or a learning design previously defined, it can be directly used in the framework. It is also important to highlight that the learning design is developed through an iterative process as the learning design knowledge used for the creation of the learning is based on a series of rules taking "*if* situation, *then* method" format, which derivate from theory, from examples or from patterns [60]. Consequently, the last step of the computer-aided framework is responsible to validate the pro cess design or can provide information for its modification, as can be seen in the Figure 9.

6.3 Step 3: Model construction and implementation

Objective: This step is intended to design, construct, solve and validate a process mathematical model.

Step 3.1: The first step is the decomposition of the mathematical model that has been provided by Step 2. The process description and the classification of the equations for its solution and further re-use in the creation of templates are the results of the (sub-) step that can be seen in Figure 11.

Step 3.2: The decomposed model is analyzed, implemented and solved in a computer-aided tool.

Step 3.3: The validation of the outputs obtained from the computer-aided tool is done by comparison with experimental data, obtained from a literature research or from dedicated experiments to create a data set for the model validation.

Note: For this step, a workflow is developed for the creation and use of a process template, as shown in Figure 5.

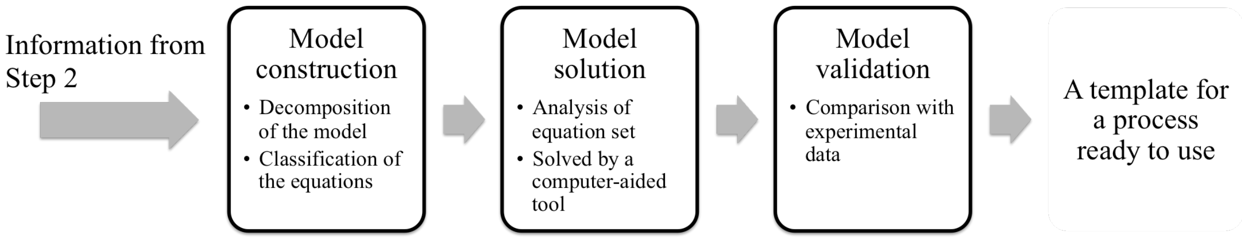


Figure 11. Workflow for the construction of a process template based on the work of Fedorova et al (2015) [61].

This workflow is based on the previous work of Fedorova (2015) [61] for the creation of template-based models. This system is further explained in Chapter 6.3.1. As result, a template for the simulation of a specific process considering the learning design is obtained at the end of the workflow.

Tools: The model is solved in a computer-aid tool, such as Python or MATLAB.

6.3.1 Concept of template-based modeling

Template-based modeling is a novel approach that requires the development of models on divisible pieces that compose a template. Therefore, a model is decomposed into three sets of equation types [62]:

- Balance equations: mass, energy and/or momentum equations.
- Constitutive equations relating intensive variables (temperature, pressure, composition) to constitutive variables (enthalpies, reaction rates, etc.).
- Connection and conditional equations relating surrounding-system connections, connections between system volumes or phases, the summation of mole fractions etc.

Furthermore, breaking the model into conceptually significant pieces allows the combination of these pieces in different ways that simplify model reuse. Each existing concept could be treated as a single building-block which could be combined with other suitable building blocks to create a new model [63]. The models can be stored in a template library.

It is important to highlight that the rational use of templates requires from the choice and therefore a previous knowledge of the user. Or, on the other hand, it can allow the creation of fictional process models by the students.

6.4 Step 4: Gamification through game-based elements

Objective: The use of game elements has been chosen as the learning tool to be integrated in the computer-aided tool based on the capability of this technique to involve the students and support an intrinsic motivation (Chapter 5).

Note: For the systematization of the gamification guidelines, it was chosen to use the Smiley model frame [64]. However, the game elements introduced are suitable to be changed by the interaction with the future users in Step 5.

In this respect, the Smiley model is an analytical framework that can be used to support the integration of game elements and learning design and it can be seen in Figure 12.

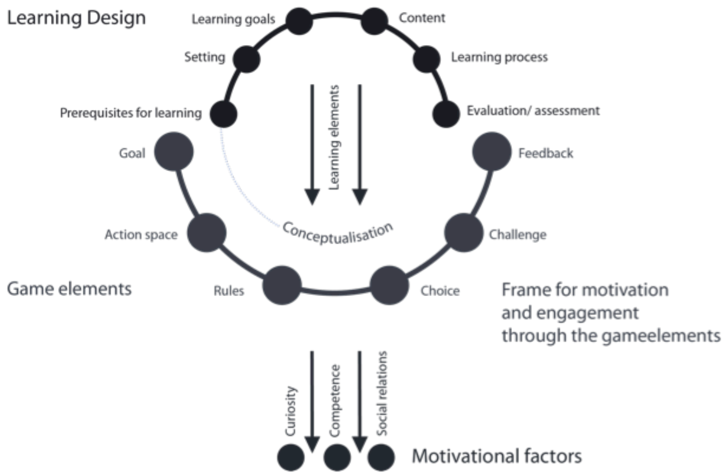


Figure 12. The Smiley model developed by Weitze (2012) [2].

Moreover, the Smiley Model (Figure 12) also incorporates in its frame the driving forces that motivates the students to reach the learning objectives in the game. Those are the motivational factors (curiosity, competence and social relations) and they need to be considered during the design of gamification.

Step: This section focuses on the definition of the different game elements that are identified for the creation of a playful environment inside the process simulator. Those game elements provide the tools for the construction of the problem context, competition, the chances, teamwork, etc. inside the platform. Figure 12 shows the six games elements that are considered however, it is important to highlight that the novelty of gamification implies the disagreement between the types and number of game elements that are defined in gamification [54], [64] and therefore, other configurations are possible.

The six game elements considered are:

- Goal. It is the objective that it is desired to achieve by the user. It can be a source of motivation. It requires being clear, structured, “un-conflicting”, and its fulfillment ideally provides a sense of competence, autonomy, and control. Additionally, it is common the creation of intermediate goals, that will enable the player to feel a series of small successes when each of the intermediate goals is reached, and increases the motivation and the curiosity of the user along the process.

- The action space enables the integration of the learning material into a gamification environment and provides the user with an easy overview and understanding.
- Rules. It helps the player to determine the effects of their choices and the effects on the learning experience. For example, a low scoring blocks part of the learning content.
- Choices should be designed to be intuitive and to encourage learning. Furthermore, choices can provide a sense of ownership to the learners and are a powerful motivational tool.
- Challenge. It enclose the learning content and therefore, it is important to highlight that the fulfilling of the challenges should provide an intrinsic motivation to the user so it continues with the learning experience [65].
- Feedback. It allows the user to gain an insight into the effectiveness of their owns efforts and it has been found crucial for the continuous motivation of the user.

Note: The design of a successful gamification requires a consideration of the learning design, and in many cases, it will also require the help of the user, thereby creating an iterative process that can be seen in Figure 9. At the end of iteration, the user can validate the gamification design or provide information that can be used for the modification of the set of game elements inside of the process simulator environment.

6.5 Step 5: Pedagogical verification

Objective: This step seeks for a confirmation of the value of the application of the methodology as a pedagogical tool and considers the feedback provided for improving the system [66] during trials of the developed learning tool.

Step: Consequently, the users test the learning tool and evaluate the gamification elements introduced in the tool, as well as, the content of the learning experience. Indeed, the computer-aided framework (Figure 9) considers an information flow for the modification of Step 2 and 4, allowing the users to be highly involved in the decisions related to the learning process and including the redefinition of the final learning and game objective.

Tools: A common choice is the use of a questionnaire for data collection that can be distributed among the users. On the other hand, an alternative can be the use of personal interviews to obtain information about how the users have experienced the learning tool although it is a more time-consuming option. It is noteworthy that the state of design of the user experience needs to depend upon the state of the platform that is tested. This will be further discussed in Chapter 7.6.

7. A case study for teaching of the aerobic batch growth of *S. cerevisiae* in glucose

In order to prove the applicability of the computer-aided framework (Figure 9), a case study is developed for the teaching and training of a fermentation case in high education, in which the target group has a previous basic knowledge about fermentation. Whereas the lack of a fermentation simulator with a thoughtful learning design has been established in Chapter 3.3, the target group is chosen considering the user need identification in Chapter 2, in which bachelor students show the highest interest for a simulator with game elements and a playful model. However, the low percentage of this group inside the survey makes this information consciously preliminary.

Furthermore, the case study chosen is related to the teaching of the aerobic growth of *Saccharomyces cerevisiae* on glucose and ethanol, presented in a batch system using the model provided by Sonnleitner and Käppeli (1985)[67]. It is noteworthy that this is not the only model for the description of this process and for example, a biochemically structured model has been proposed by Lei *et al* (2001) [68]. However, a kinetic model with a lower level of detail is more suitable to the target group.

7.1 Case Study background

Saccharomyces cerevisiae is the principal yeast utilized in the traditional processes of the biotechnological industry, such as beer production, and in the modern production of, for example recombinant proteins [69]. This is based on its unique physiology, the well-established methods for its genetic manipulation, and its possibility to be cultivated under aerobic and anaerobic conditions, which allows the optimization of the production techniques for a specific fermentation process with *S. cerevisiae*. All these characteristics make *Saccharomyces cerevisiae* a key organism for the biotechnological industry.

More specifically, this case study focuses on the teaching of the aerobic batch growth of *S. cerevisiae* in a stirred tank reactor with glucose as carbon source. The batch process is one of the most common operation modes in the traditional fermentation industry due to its simplicity, while the stirred tank reactor is one of the most frequently found reactor configurations that allows an easy control of the conditions inside the reactor. Furthermore, this is a typical configuration used to promote the growth of microorganisms and the presence of oxygen will have a higher yield of microbial growth than the fermentation. Moreover, the aerobic growth of *S. cerevisiae* has a mixed metabolism with fermentation and respiration, called Crabtree effect [70], and it provides the opportunity to show both glucose degradation routes.

More information about *Saccharomyces cerevisiae* and this process is presented in Appendix 4; which embed the learning content of the platform developed in the second interaction of the framework.

This process covers several important concepts and can be helpful in the training of the decision-making skills of students.

7.2 Step 1: Need identification

The learning goal involves the acquisition of knowledge about all the steps required for the description of the aerobic cultivation of *Saccharomyces cerevisiae* in glucose and its by-product ethanol in a stirred batch tank reactor.

In order to establish the need related to Step 1.1, a survey was done with 10 students of the 2nd year bachelor in Sustainable Biotechnology who fulfill the requirements of the target group, as it is feasible to consider that the students have a previous elemental knowledge about the fermentation process and about microorganism growth kinetics. Furthermore, these students will participate on the pedagogical verification (Step 5). In the need identification survey, it was asked:

(1) If the student has ever seen a medium or large scale bioreactor (Q1),
(2) If the student was interested in the process of bioconversion (Q2),
(3) If the student was able to choose between a continuous and discontinuous process (Q3),
(4) If the student lacked the possibility of easily exploring different kinetic scenarios (Q4), and
(5) If the student was familiar with the metabolism of *S. cerevisiae* growing on glucose under aerobic conditions (Q5).

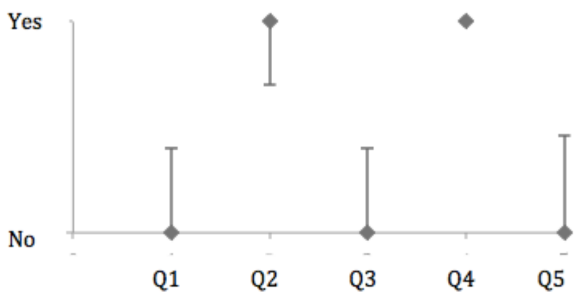


Figure 13. Survey for the need identification in the application of the developed framework

As it can be seen in Figure 13, it was found that the 80% of students have never seen a medium-large bioreactor (Q1), whereas the same percentage of participants were interested in the bioconversion process (Q2). On the other hand, 80% of the participants were lacking of decision-making confidents in the choice of important process definition, such as continuous versus discontinuous operation (Q4). Finally the need was identified as 100% of participants missed the possibility of explore fictional and non-fictional scenarios, while 80% recognized that they didn't have any previous knowledge of the kinetic model of *Saccharomyces cerevisiae*.

Therefore, from the information obtained in the Step 1, it is identified learning goals such as knowledge about the kinetic of aerobic growth of *Saccharomyces cerevisiae* in glucose and skills in design decision-making.

7.3 Step 2: Process Description

As it is mentioned in the chapter 6.2, in this step it is described the pedagogical and mathematical perspective of the teaching of the aerobic growth of *Saccharomyces cerevisiae* on glucose and ethanol, presented in a batch system.

Initially, the mathematical description of the process is done based on the previous works of Sonnleitner and Käppeli (1985) and Fernandes *et al.* (2013) [67], [71]. This model is a complete described process that provides an accurate representation of the process with well-established constants and biological parameters.

1. This model describes the glucose-limited aerobic growth of *S. cerevisiae* with an overflow metabolism and predicts the concentrations (in g/L) of glucose, ethanol, biomass and oxygen. Moreover, the model includes the inhibition of the consumption of ethanol due to high concentration of glucose.
   Specifically, the model relies on three stoichiometric reactions describing the growth of biomass on glucose by respiration (Eq. 1) and by fermentation (Eq. 2), as well as the growth of biomass on ethanol by respiration.

$$\mathrm{C_6H_{12}O_6} + a\ \mathrm{O_2} + b\ 0.15[\mathrm{NH_3}] \rightarrow b\ \mathrm{C_1H_{1.79}O_{0.57}N_{0.15}} + c\ \mathrm{CO_2} + d\ \mathrm{H_2O} \tag{1}$$

$$\mathrm{C_6H_{12}O_6} + g\ 0.15[\mathrm{NH_3}] \rightarrow g\ \mathrm{C_1H_{1.79}O_{0.57}N_{0.15}} + h\ \mathrm{CO_2} + i\ \mathrm{H_2O} + \mathrm{j}\ \mathrm{C_2H_6O} \tag{2}$$

$$\mathrm{C_2H_6O} + l\ 0.15[\mathrm{NH_3}] \rightarrow l\ \mathrm{C_1H_{1.79}O_{0.57}N_{0.15}} + m\ \mathrm{CO_2} + n\ \mathrm{H_2O} \tag{3}$$

2. The bioconversion of glucose and ethanol is done at 30°C, pH 4 and a flux of air of 1 vvm.
3. This model consider the 3 different metabolic pathways
   a. Oxidation of glucose to biomass.
   b. Reduction of glucose to biomass.
   c. Oxidation of ethanol to biomass.
   d. Oxygen supply.
4. The complete kinetic model can be found in the previous work of Sonnleitner and Käppeli (1985) [67] and Fernandes *et al.* (2013) [71]. In Table 6 it can be found the kinetic expression for the description of the process.
5. The main assumption of the model is that the production of acetate and glycerol can be considered irrelevant for simplification purposes. Furthermore, the stirrer speed is also assumed to be constant and spatial distribution of concentrations and temperature are not considered.
6. The values of yields, stoichiometric coefficients, biological parameters, design conditions and initial concentrations can be found in Table 4.
7. The collected model is further developed in Step 3.

<u>Step 2.2</u>: The learning design created for the specific case study is collected in Table 5. It is noteworthy that this learning design have similar learning goals that the 3rd Semester subject of Kinetics and Modeling of Bioprocess in the bachelor of Sustainable Biotechnology in Aalborg University in Copenhagen [72], but with

different settings. Therefore, theoretically, the resulting simulator of the application of the methodology can be used as a complementary tool for the teaching of this subject.

Table 4. Values of the yields, stoichiometric coefficients, biological parameters, design conditions and initial concentrations.

Symbol	Value	Ref
Yields		
Y_{XG}^{Ox}	0.8 $\frac{g\ G}{g\ X}$	[67]
Y_{XG}^{Red}	0.05 $\frac{g\ G}{g\ X}$	[71]
Y_{XE}	0.72 $\frac{g\ E}{g\ X}$	[67]
Stoichiometric coefficients		
Y_{OG}	1.067 $\frac{g\ O}{g\ G}$	[71]
Y_{EG}	0.45 $\frac{g\ E}{g\ G}$	[71]
Y_{OE}	2.087 $\frac{g\ O}{g\ E}$	[71]
Biological Parameters		
q_G	3.5 $\frac{g\ G}{g\ X \cdot h}$	[67]
q_0	0.37 $\frac{g\ O}{g\ X \cdot h}$	[71]
q_E	0.32 $\frac{g\ E}{g\ X \cdot h}$	[71]
K_G	0.17 $\frac{g\ G}{l}$	[71]
K_0	0.0001 $\frac{g\ O}{l}$	[67]
K_E	0.56 $\frac{g\ E}{l}$	[71]
K_I	0.31 $\frac{g}{l}$	[71]
t_{lag}	4.66 h	[71]

Symbol	Value	Ref
Aerobic conditions		
$C_{O_2}^*$	0.0075 $\frac{g\ 0}{l}$	[71]
kla	1004 h^{-1}	[71]
Initial concentrations (For the experimental validation)		
$C_{G\ (t=0)}$	18 $\frac{g\ G}{l}$	
$C_{O_2(t=0)}$	0.00755 $\frac{g\ O}{l}$	
$C_{E\ (t=0)}$	0.34 $\frac{g\ E}{l}$	
$C_{X\ (t=0)}$	0.1 $\frac{g\ X}{l}$	

Table 5. Learning design, created for the specific case study.

Learning elements	Description
• Prerequisites of learning	• Previous elemental knowledge about the fermentation process and microorganisms growth kinetics.
• Learning goal	**Knowledge** • Understand the effects behind the aerobic growth of *Saccharomyces cerevisiae* in glucose and in ethanol in a stirrer tank batch. • Understand the mathematical model. **Skills** • Evaluation of "what if" scenarios based on the modification of kinetic and biological parameter. **Competences** • Meaningful decision about the choice of microorganisms, mode of operation and configuration, the conditions of the culture.
• Setting	• A computer-aided implemented and designed by the author, although the first iteration was designed a paper prototype.
• Content (more information in Appendix 4)	• Covers theory of the fermentation process further than the specific case study. • Introduction to kinetic and modeling of bioprocess. • The kinetics of the aerobic growth of *S. cerevisiae* in glucose and in ethanol in stirrer tank in batch.
• Learning process	• Kolb's experiential cycle based on the theory explained in Section 2.2. For example, the user is encourage to modified biological parameters from which he/she is chosen one and with the change of the value a new plot of the kinetic model is generated. Therefore, the student/user can developed an abstract concept and test its own hypothesis.
• Evaluation/assessment	• Based on the learner's activity in the setting and the Kolb's experiential cycle. The questionnaire will have variable feedback and the learning design of the question varies between constructivist and instructionism. On the other hand, the evaluation of "what if" scenarios follows a Kolb's experiential cycle, with the trail of hypothesis and abstract conceptualization. This can be further seen in Chapter 9.

7.4 Step 3: Model construction and implementation

For the specific case of fermentation, a sub-workflow for the construction of the process was developed and it is shown in Figure 14.

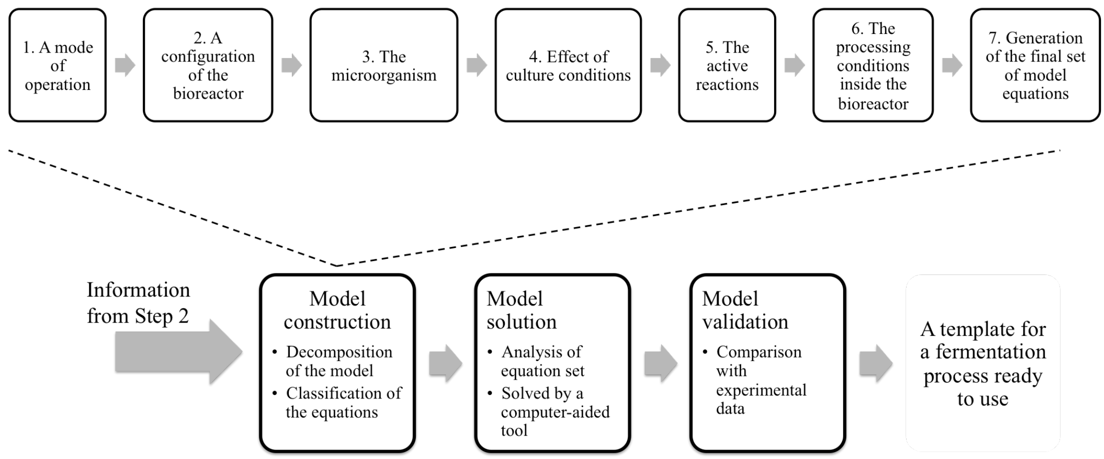


Figure 14. Workflow for the model construction of a fermentation.

<u>Step 3.1</u>: Figure 14 is an expansion of Figure 11, focusing on the construction of a model of a bioconversion process, including the decomposition of the model and the subsequent classification of the equations using the upper part of Figure 14 as well as related to the learning content.

As it was been previously mentioned, the types of equations are classified for the construction of models into:

Balance equations

Mass balance: $\frac{d\,(V_L(t)C_X(t))}{dt} = \mu(t)V_L(t)C_X(t)$

Due to the lack of time, it was not possible to implement the balances corresponding to the energy and the momentum conservation.

Constitutive equations

In Table 6, the mathematical equations that describe the different processes that are involved in the aerobic growth of *S. cerevisiae* on glucose can be seen, based on previous work by Sonnleitner and Käppeli (1986) [67].

None connection or conditional equations were included in the process description.

Table 6. Kinetic expressions for the description of the aerobic growth of *Saccharomyces cerevisiae.*

Process	Equation
Oxidative capacity of the cells (r_o)	$r_o = q_o \cdot \frac{C_{O_2}}{C_{O_2} + K_0} C_X$
Glucose uptake rate (r_G)	$r_G = q_G \cdot \frac{C_G}{C_G + K_G} C_X$
Product formation rate (r_p)	$r_p = q_p \cdot \frac{P}{P + K_P} C_X$
Oxidation rate of glucose (μ_G^{Oxid})	$\mu_G^{Oxid} = Y_{XG}^{Oxid} \frac{1}{Y_{OG}} \Bigg(\min \Bigg(q_o \cdot \frac{C_{O_2}}{C_{O_2} + K_0} \Bigg), Y_{OG} q_s \frac{C_G}{C_G + K_G} \Bigg)$
Reduction rate of glucose (μ_G^{Red})	$\mu_G^{Red} = Y_{XG}^{Red} (q_G \frac{C_G}{C_G + K_G} - \frac{1}{Y_{OG}} \Bigg(\min \Bigg(q_o \cdot \frac{C_{O_2}}{C_{O_2} + K_0} \Bigg), Y_{OG} q_G \frac{C_G}{C_G + K_G} \Bigg)$
Oxidation rate of ethanol (μ_E)	$\mu_E = Y_{XE} \Bigg(\min \Bigg(q_o \cdot \frac{C_{O_2}}{C_{O_2} + K_0} \Bigg) - \min(k_o \cdot \frac{C_{O_2}}{C_{O_2} + K_0}, Y_{OG} q_G \frac{C_G}{C_G + K_s}), Y_{OE} q_E \frac{C_E}{C_E + K_E} \frac{K_i}{C_G + K_i} \Bigg)$
Biomass growth rate (μ)	$\mu = \mu_G^{Oxid} + \mu_G^{Red} + \mu_E$

The rates of consumption and production of glucose, ethanol, oxygen and biomass can be described with a process matrix. Therefore, the rates for each component can be obtained by multiplying the transpose of the stoichiometric matrix (Z') with the process rate vector (ρ). Table 7 shows the model matrix that was implemented in the computer-aided solver.

Table 7. Process matrix describing the conversion rates and stoichiometry for each model variable: glucose, ethanol, oxygen, and biomass.

Components→ Processes↓	C_G	C_E (Z)	C_O	C_X	Rate vector (ρ)
Biomass growth by glucose oxidation	-1	0	-Y_{OG}	-Y_{XG}^{Ox}	$\frac{1}{Y_{OG}} \Bigg(\min \Bigg(q_o \cdot \frac{C_{O_2}}{C_{O_2} + K_0} \Bigg), Y_{OG} q_s \frac{C_G}{C_G + K_G} \Bigg) C_x$
Biomass growth by glucose reduction	-1	Y_{EG}	0	Y_{XG}^{Red}	$q_s \frac{C_G}{C_G + K_G} - \frac{1}{Y_{OG}} \Bigg(\min \Bigg(q_o \cdot \frac{C_{O_2}}{C_{O_2} + K_0} \Bigg), q_s \frac{C_G}{C_G + K_G} \Bigg) C_x$
Biomass growth by ethanol oxidation	0	-1	-Y_{OE}	Y_{XE}	$\left(\frac{1}{Y_{OE}} \right) \cdot \Bigg(\min \Bigg(q_o \cdot \frac{C_{O_2}}{C_{O_2} + K_0} \Bigg) - \min(q_o \cdot \frac{C_{O_2}}{C_{O_2} + K_0}, Y_{OG} q_s \frac{C_G}{C_G + K_G})), Y_{OE} q_E \frac{C_E}{C_E + K_E} \frac{K_i}{C_G + K_i} \Bigg) C_x$
Oxygen supply	-	-	1	-	$k_L a \, (C_{O_2}^* - C_{O_2})$

Furthermore, it is not integrated the chemical (pH modeling) and physical (mass transfer) models. However, matrix notation can be extended with the integration of the chemical and physical models as it has been done for *Streptomyces coelicolor* in the previous work of Gürkan Sin (2008) [73]. This further consideration is mentioned in Chapter 10.2.

Step 3.2: The decomposed model was implemented as a computational problem and solved within Python 3.6, and can be seen in Appendix 1. The model is implemented using object-oriented programming. This allows the use of inheritance for code reuse and extensibility in the form of classes or modules. However, the user only interacts through a visual interface, which the first prototype will be further show in Chapter 9.

Step 3.3: Finally, the validation of the model was done by the comparison of the model output with experimental data, which can be seen in Figure 9, and literature data [67], [71]. Furthermore, the experiment was performed following the protocol provided in the Appendix 2.

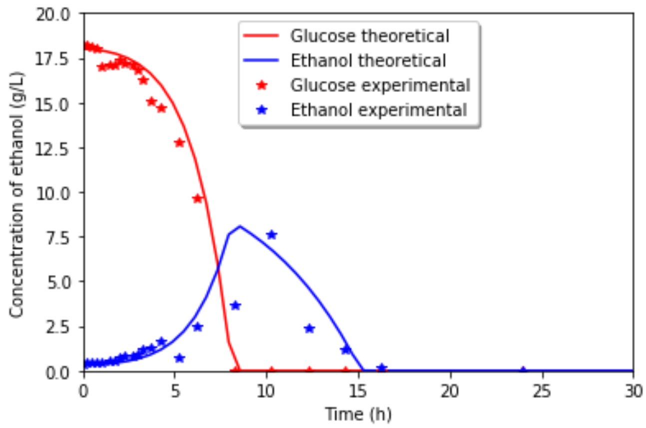


Figure 15. Comparison of the model output with experimental data used for model validation. The small differences between the model and the experimental data are evaluated as acceptable, i.e. the model describes the data sufficiently well.

It is noteworthy that the overflow metabolism is found in other biological systems like in bacterium *E. coli* (with the acetate switch instead of ethanol) or in other Crabtree positive yeasts [74], and therefore it should be possible to modify this model to other biological systems. However, due to the limited time of the project, this possibility has not been further explored.

7.5 Step 4: Gamification through game-based elements

As mentioned in Section 6.4, gamification is usually developed in iterative processes in collaboration with the future users of the learning technology. In spite of thorough literature research, we have not found many references that provide general advice on how to integrate game elements in a tertiary educational computer-aided software. Therefore, the integration and validation of the game elements in the platform was done in collaboration with the users and can be seen in Table 8.

Table 8. Gamification in the case study.

Game element	Description
• Goal	• Initially, the game goal is the earning of a job position based on a score in the questionnaires. This game goal differs from the learning goal, presented in Table 5.
• Action space	• The idea of getting a job also provides the software with an action space, since the learner is considered to participate in a recruitment process.
• Rules	• The user needs to answer a series of questions, in which the learner proves to have the knowledge required, and each correct answer has a 1-point reward. Only with a minimum number of points the game is complete. Moreover, the rules are extended with the development of the platform and with the addition of a new activity; a new set of rules is included. This is shown in Chapter 10, with the implementation of a set of rules for a mini-game.
• Choice	• The user can choose the display of learning hints or to answer or no to answer the questions. Furthermore, the learning process allows failing and provides the possibility to review the redundant knowledge faster, while the hints can give a deeper insight to the theoretical background. On the other hand, parameters and kinetic equations are easily modified. This provides the opportunity of checking "what if" scenarios and how decisions regarding the reactor operating conditions affect the system, and in this way one can stimulate the user in the development of a more critical way of thinking.
• Challenge	• It is provided by a structured series of questions and a mini-game. More specifically, the questions are commonly multi-choice or require the introduction of a numeric solution and only the correct choice allows the user to increase the complexity level of the questions and to earn the points necessary to fulfill the game goal.
• Feedback	• It needs to be variable, positive and immediate in order to enable the student to understand the connection between cause and effect, and provide guidance and information. It is based on the previous work of Weitze (2016) [64].

7.6 Step 5: Pedagogical verification

The pedagogical verification was done through a user experience of the target group employing a questionnaire in order to verify the pedagogical value of the paper prototype and to implicate the users in an early stage of the design. The questionnaire used after the learning experience can be found in the Appendix 3.

The learning experience was done using a paper prototype (Figure 16) with 10 students of the 2nd year of the bachelor in Sustainable Biotechnology in Aalborg University Copenhagen. The use of a paper prototype allows the student to provide inputs in the design of the platform that could be immediately implemented during the trials. On the other hand, the low number of participants allows a close collaboration between the developer and the future user; whereas it has been found than 85% of the of all usability problems are found by an average of 5 users [75].

The paper prototype (Figure 16) is based in the use of multi-choice questions and with the same system for the choice of the definition of the process as is implemented in FermProc (in Chapter 9.2).

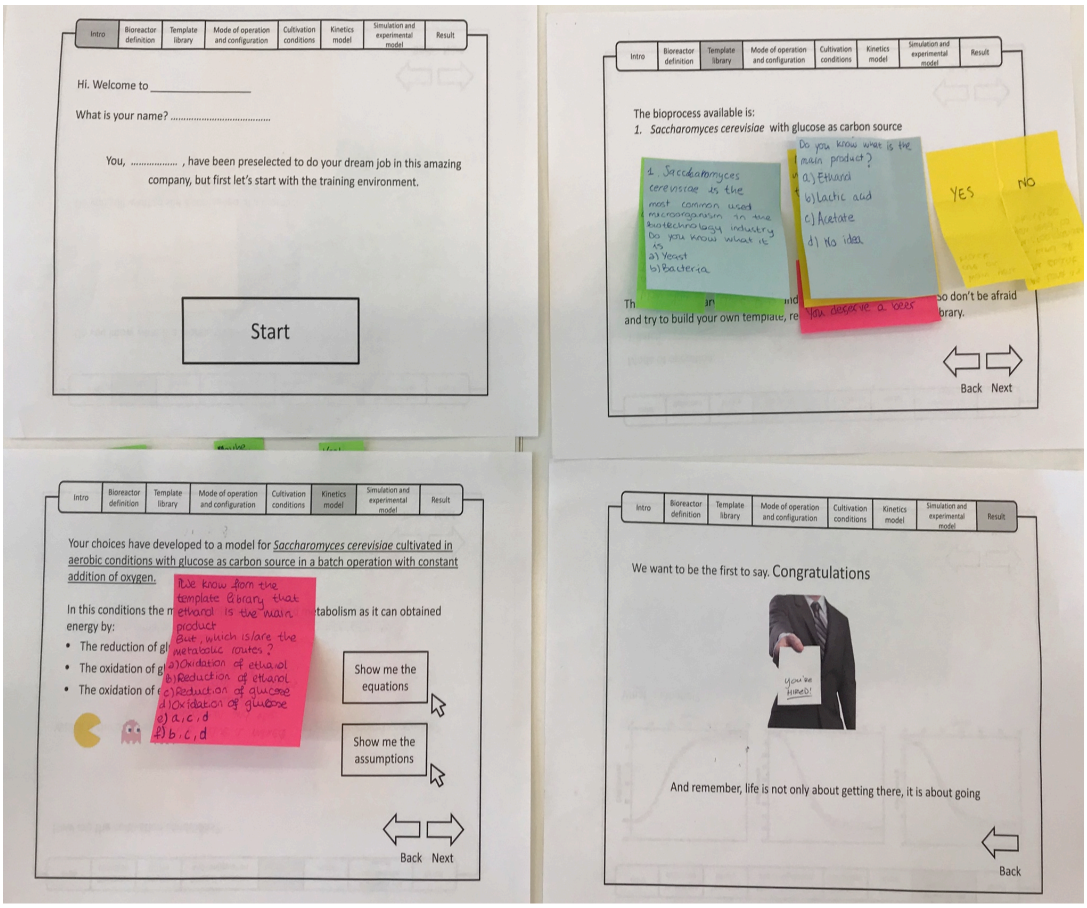


Figure 16a). First paper prototype in which was implemented the case study.

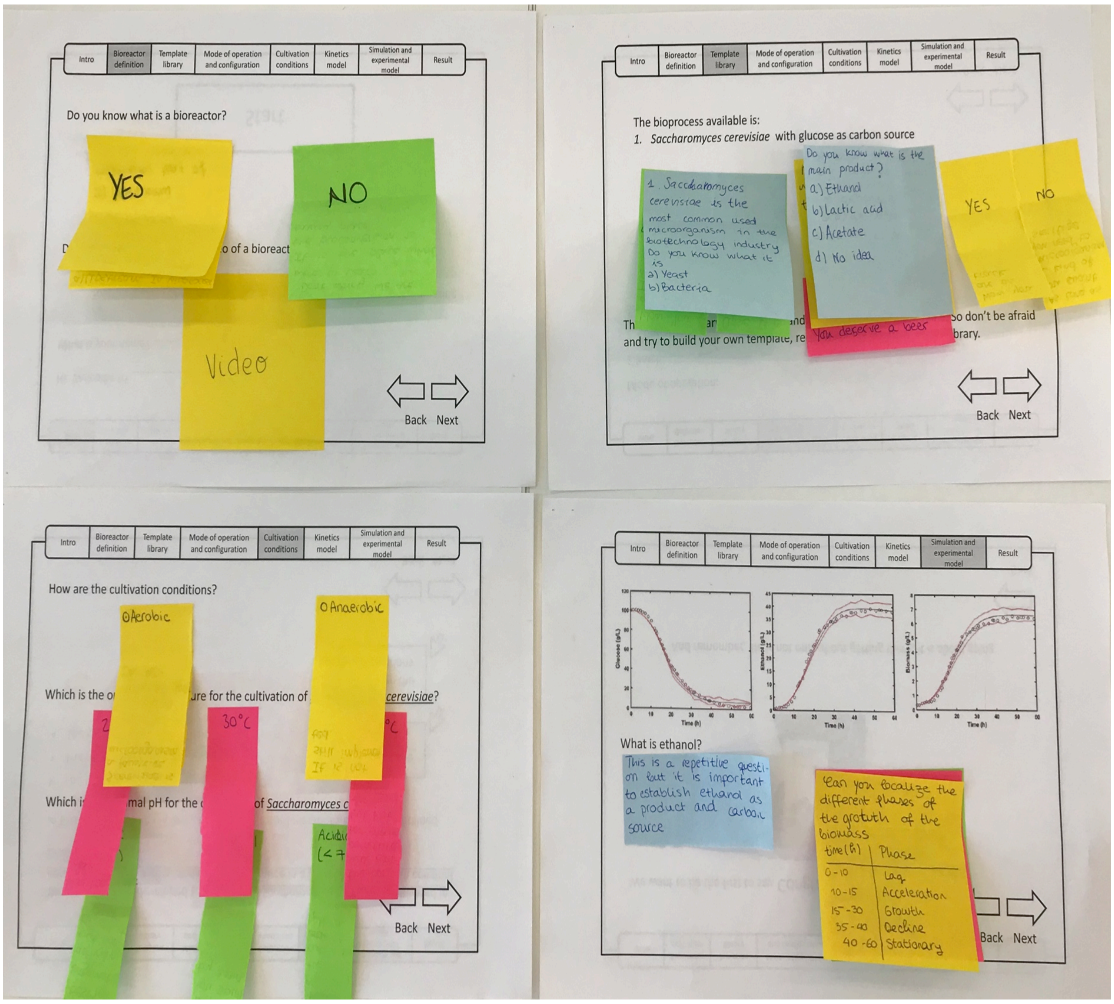


Figure 17b). First paper prototype in which was implemented the case study.

The information gathered in the learning (user) experience is presented and deeply analyzed in following chapter.

8 Assessment of the data obtained in the learning experience

As it is mentioned in the previous chapter, from the pedagogical verification step done by 10 students belonging to the target group is collected quantitative and qualitative data. This data is further presented and analyzed in this chapter with the aim of an early validation of the methodology proposed in this thesis. It is important to highlight that the few collected data for only one case study make the validation of the methodology preliminary.

8.1 Quantitative data

The quantitative data obtained from the anonymous questionnaire after the user experience can be seen in Figure 17. In this graph, 100% of the students agreed with the usefulness of this tool for the learning of fermentation, from which 30% totally agreed. On the other hand, 80% of the participants agreed in that it could be a good idea for studying, although this opinion showed a higher standard deviation among the students. Furthermore, when it is asked about the usefulness of the tool to extrapolate knowledge to a "hands on" experience, 80% agreed while only 10% disagreed. Also, to evaluate the level of difficulty perceived by the user when confronted with the tool, it is asked if the content was easy and 30% agreed, while 70% answered neutrally. Finally, the feedback provided by the platform as a game element is evaluated and it could be concluded that the users liked to receive positive feedback, with 40% that totally agreed, 50% that agreed and 20% had a neutral opinion. Meanwhile, in the paper prototype, the design of the interface was not so attractive to 14% of the users, and very attractive to 28% of the respondents to the survey.

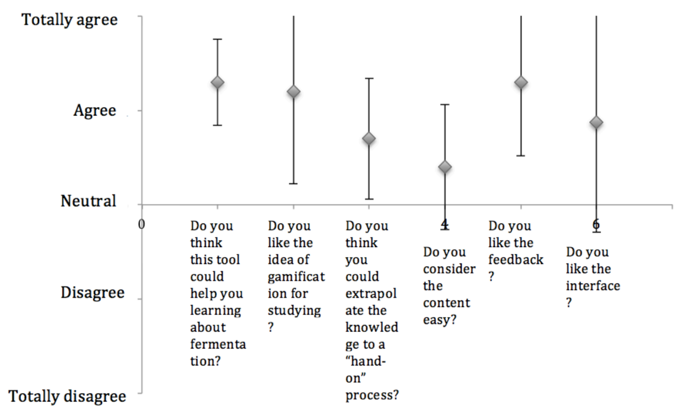


Figure 18. Survey about the usability and enjoyability of the prototype from a class (10 students) of the second year of the bachelor in Sustainable biotechnology.

Moreover, in order to increase the co-design relation between future users and the designer, in the questionnaire of Appendix 3, it is asked the students which area they would tackle to improve the game and it was evaluated in a 100% scale as can be seen in Figure 18. From Figure 18, it can be concluded that users did not prioritize a more attractive interface but to make the platform more challenging, increasing the number of tasks and the content. In average, the participants also asked for a clarification of the game objectives and therefore in the next interaction of the framework it is added rules as well as hints with the corresponding theory behind each level.

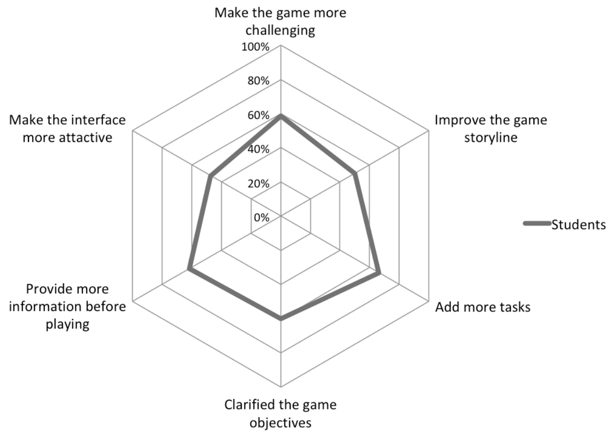


Figure 19. Survey about the future perspective and improvement focus of the platform "FermProc".

Therefore, although the design of the interface was one of the questions that showed a higher level of disagreement between the participants in Figure 17, it was not prioritized as the students considered the platform as a valuable tool for learning and they gave more importance to the learning progress in Figure 18. Then, in the next implementation of the game is considered an increase in the number of hints and the links to the theory, providing a clear set of rules and planning the addition of extra activities, such as a guess-who?? (bioreactor version) shown in Chapter 9.2.

8.2 Qualitative data

Apart from the quantitative data obtained, some of the questions were also given extra space where the participants could write down their opinion about the different attributes of the platform as it can be seen in the Appendix 3. This yielded responses about the usefulness of the platform such as "*It helps to understand how many parameters have to be optimal in order to achieve the maximum growth/production in fermentation"* or "*The questions force you to think about the answer/possibilities in a good way so you learn it*" or *"I think it is really helpful with its visual content. It makes the user to get a real overview of the process and it is more enjoyable than studying from a book full of text with no schemes or pictures"* or "*the explanations are nice if you are a beginner*". When the participants were asked about how the tool could help them to extrapolate they knowledge provided by the prototype into an actual fermentation they comments think like "*you can imagine the outcome of a process better"* or "*you could use the information to set the optimal conditions"* other participant thought about other possibility such as "*It might help you on your exam where you need to explain briefly and clearly this process or maybe for an oral presentation*".

Furthermore, the opinion about the feedback can be summarized with one of the comments "*It's psychologically tested that you performance better under positive feedback"*. On the other hand, the opinions the design of the interface shows more variety with comments like; *"I like the interface as it looks very 90's, however I will add round-edged buttons and more fancy arrows"* while another believed that "*it was a bit simple and uninspiring"* and another one wrote that *"the design looks very professional"*.

Meanwhile, when it was asked some inputs for the improvement of the software, some of the opinions were given such as "*It could be great to get more pictures/diagrams of the bioreactor*" or "*Well, it is good, but as a student I would like to see hints there. You could also make it more competitive for example; you can somehow introduce the multiplayer. People like competitive games more.*" or "*I really think students could benefit from this because of the explanations, and the experts might find the chance to choose the parameters very useful.*" And "*do not add <u>I don't know</u> button, make people think and make a guess. In anyway whether the answer was correct or not, you could make the explaining feedback. You could also introduce a system of hints, for example, you can choose some facts about the species, product, reaction kinetics, etc.*"

8.3 Overall conclusions of the learning experience

Based on the quantitative data it can be concluded that 100% of the student participants found useful the tool developed based on the methodology and the participants encouraged the process of co-design and getting involved in the development of the platform by providing critical and helpful feedback. Among this feedback, the students commented and prioritized for an increase in the level of the platform, as well as maintained the positive feedback. On the other hand, the opinions about the design of the interface showed the higher qualitatively and quantitatively variation amongst peer. However, it is important to highlight that all measurements, especially of opinions and in a reduced group, are subject to fluctuating that can affect the data's reliability and validity. Therefore, it is highly recommended to perform a second learning experience. Despite this, the reduced learning experience allows a stronger co-creation of the platform and it was easy to address the main concern of the future users in the limited time available. This resulted in the addition of learning hints about and the creation of a video to show bioreactor, etc. and it is further presented in Chapter 9. It is important to highlight that the modifications proposed here didn't require the modification of the learning and gamification design (Table 4 and 5) developed in Chapter 7.

9 FermProc. A software platform for the teaching of the case study

FermProc is the resulting tool from the implementation of the developed computer-aided modeling framework from Chapter 7, and it considers the information presented in Chapter 8. In this Chapter, the software architecture created for FermProc that integrates the learning design and the creation of model templates is briefly presented and the main challenges of the design of the platform and the reason behind its design in Python are explained. Furthermore, a brief overview of the current state of the platform is presented.

It is important to highlight that FermProc is still in development.

9.1 Software architecture for FermProc

Application software architecture serves as the blueprint for individual application systems and their interactions. For FermProc, a software architecture structured around object-orientation was created and it was based on the division of responsibilities into objects, each containing the data and behavior of the object [76].

FermProc has been initially created as a desktop application. This was done due to the need of a short development time [77]. However, in the future, it is expected to work online as well as in the desktop.

Figure 19 shows the software structure developed for FermProc.

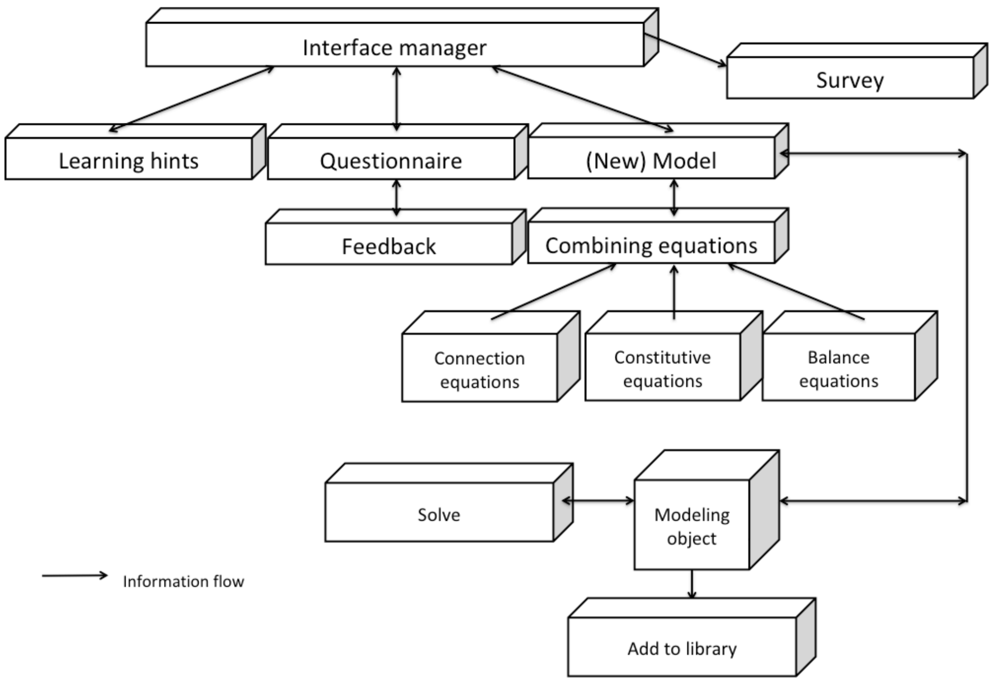


Figure 20. Schematic software architecture for FermProc.

In Figure 19, the connections between the interface manager (GUI) and the three interactive layers of the software correspond to a text format of learning hints, questionnaires, and the process model. The learning hints are only interacting with the user manager interface with the opening and closing of the window or the reproduction of multimedia resources. Meanwhile, questionnaires have two layers as the display of the feedback depends on the input sent by the user manager. On the other hand, the model is connected to the user interface for its selection and modification. Further, the model is composed by the combination of equations and is established as a modeling object that solves and/or adds to the template library, as it has been mentioned in Chapter 6.3.1. Finally, the interface manager integrates a survey that sends information to the programme console (and in the future to a server), for the integration in the same platform of the pedagogical validation. However, this last process is still in its prototype phase

FermProc was written in the Python programming language. Python is an *interpreted, interactive, object-oriented* programming language [78], and allows the creation GUI of FermProc with PyQt toolkit and more information about this choice is mentioned in Chapter 9.1.2. However, initially, the challenges that were faced during the creation of FermProc are explained.

9.1.1 FermProc challenges

Due to its pedagogical aim and its conceptual design, FermProc must be reliable, user-friendly, able to provide the user with an enjoyable learning experience, and with an easy system for the addition of new models or modifications of the model implemented. The main challenges of this platform are:

- To provide an organized and free-flowing learning experience. To solve this problem, clear directions are supplied in the platform.
- It must allow and encourage the modification of the model. To do so, the parameters that can be modified are displayed and the kinetic tendency and learning hints are also included to facilitate the experiential learning.
- Feedback must be intrinsic and variable. This is an important element for the implementation of the learning design and gamification. Using one question as an example:

  When you choose a production organism, the best choice is:

  1. An organism, which can only function under aerobic conditions.
  2. An organism, which can only function under anaerobic conditions.
  3. <u>An organism, which can only function under aerobic conditions and anaerobic conditions.</u>

  Although this question is not a challenge for the target group users, it promotes reflections about the different considerations that need to be taken into account and intrinsic feedback is provided to take the knowledge a step ahead.

  Feedback: Aerobic growth can be convenient for biomass production (previous to product formation), because the growth rate is generally much higher. Meanwhile anaerobic growth can give higher yields and lower process costs due to the absence of oxygen required in the process. Therefore it can be useful to have an organism that can grow both aerobically and anaerobically. For example, in the case that you want to perform fermentation in which you separate a growth phase (batch, aerobic) and a production phase (feed phase, anaerobically). Can you think of an example? Do you know how beer is produced? Maybe ask the person next to you…

This learning follows the structure of Figure 5, which corresponds to a constructive learning design. Also, the last sentence invites a collaborative learning, although it has not been integrated into the platform as a chat or other communication system due to the limited time. On the contrary, some of the questions are still using an instructionism approach such as:

What is fermentation?

1. The process where enzymes convert organic substrates to useful products. This process is either aerobic or anaerobic.
2. The conversion of organic substrates by microorganisms. In the classical definition, this process is anaerobic but the definition is also used wider, including aerobic processes.
3. The anaerobic conversion of fossil feedstock by microorganisms.
4. The conversion of fossil feedstock by enzymes. In the classical definition, this process is anaerobic but the definition is also used wider, including aerobic processes.

And the correspond extrinsic feedback is:

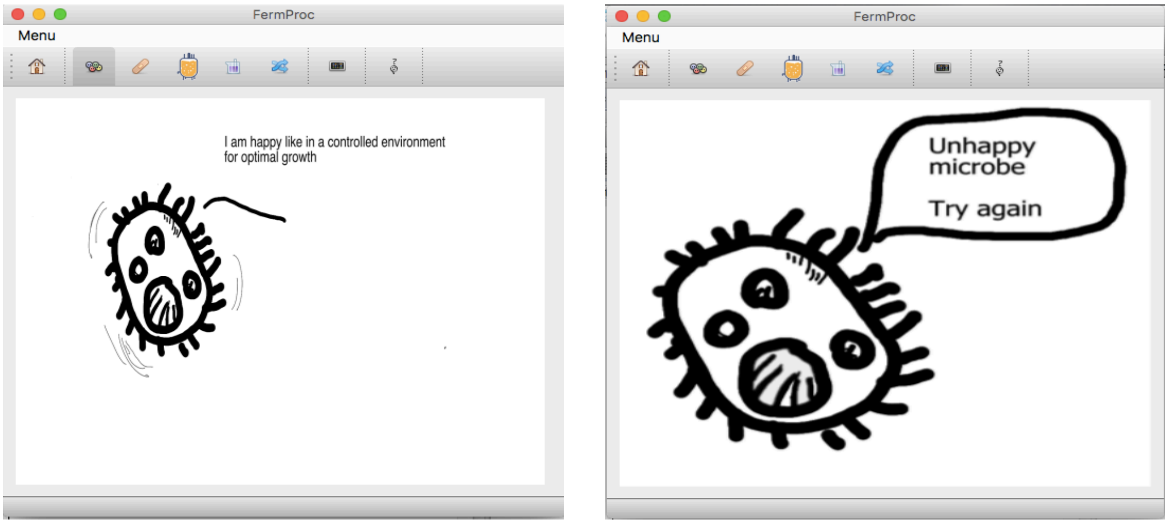


Figure 21. On the left, the positive extrinsic feedback in which a microorganisms is saying "*I am happy like in a controlled environment for optimal growth*" and on the right, negative extrinsic feedback.

It is important to highlight that the platform has been designed with a constructivist learning design aimed and therefore this feedback will be modified into an intrinsic feedback.

9.1.2 Why Python?

Although the majority of software presented in Table 2 were developed in Java, this software has been implemented in Python as, during the past decade, Python has become the de facto standard for exploratory, interactive, and computational-driven scientific research [79]. Furthermore, Python allows rapid development and prototyping and [80]:

1. It can be used interactively and does not need to be compiled.

2. It has a simple and very clear syntax.
3. It is an "open source" project with "a free software license".
4. It has bindings for most of the compiled languages, like C/C++ and Fortran.
5. It includes rich collections of packages, which implement basic actions, numerical methods, etc.

Based on these advantages, the programming language selected was Python with the use of the packages collected in Table 9:

Table 9. Main Python packages used in FermProc.

Package	Description
Sys	System-specific parameters and functions. It provides a summary of the available constants, functions and methods.
PyQt5	Binding for the Qt application framework
Matplotlib	Python 2D plotting library
Scipy.integrate	Package for integration and ODEs
Math	Fundamental package for the use of mathematical expressions
Numpy	Fundamental package for the use of vectors and matrix
CX Freeze	It converts Python scripts into executable (.exe)

9.2 An overview of FermProc

In this section, a series of indications for the use of FermProc are collected. Furthermore, the applications have a menu and a toolbar that allows users to see the instructions and play with the application. The theory implemented in the software is explained in Appendix 4.

Once the software is executed, the first screen appears (Figure 21).

Figure 22. Preview of the initial screen of FermProc.

In this screen, two possibilities are available:

- Insert a new template. This option cannot be executed yet and therefore, the platform will inform the user that it is not available.
- Reuse a template.

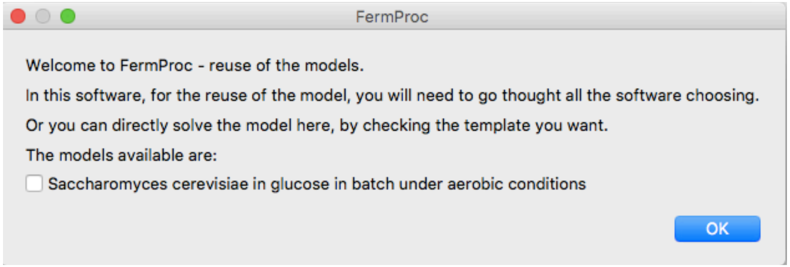


Figure 23. Screen for the reuse of model templates.

When the user checks the model, it is automatically soved. On the other hand, the user can go through the game and select the conditions for the generation of the kinetic model. The current version of FermProc has only one kinetic model and therefore there is no freedom for the design of the fermentation. Further in this explanation, the model will be "selected" by the choice of the conditions.

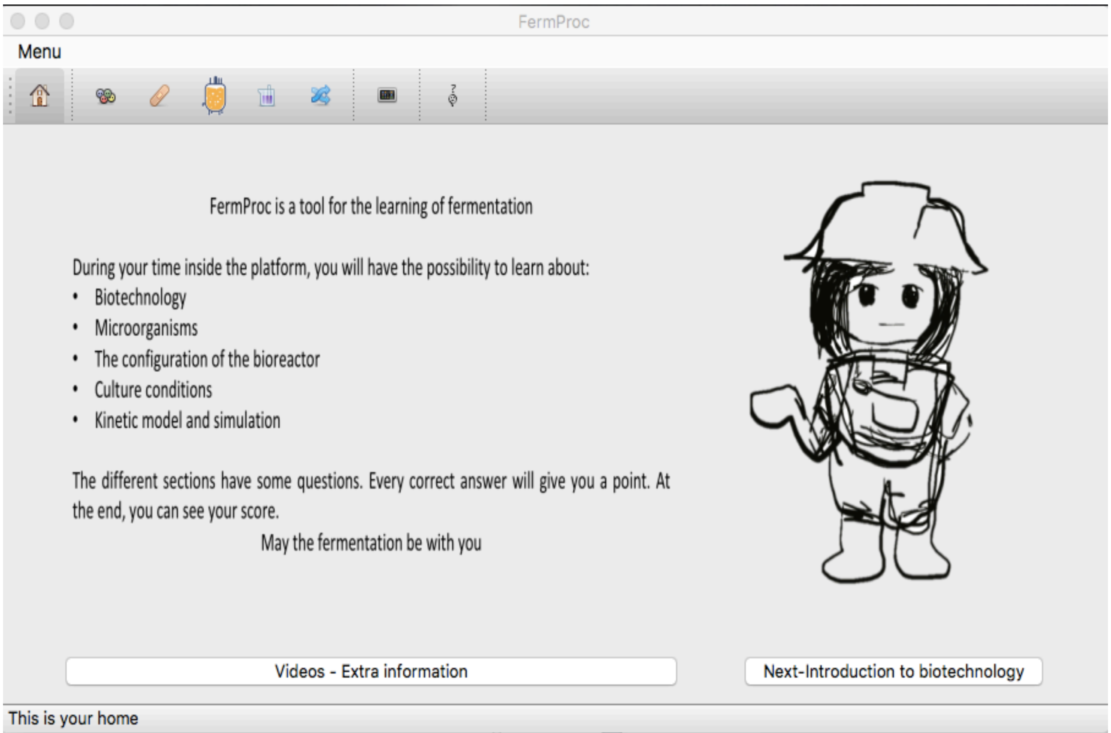


Figure 24. Home screen

This first screen (Figure 21) sends the user to "Home", which corresponds to Figure 23. In this screen, the main points of the theory of the current version of FermProc are established (and are further explained in Appendix 4) and two buttons connect with two possibilities:

- Obtain extra information by multimedia resources. The platform has integrated videos for the dissembling of a bioreactor and the preparation of medium on a laboratory scale. Those videos can also be accessed by clicking on the toolbar ().
- Or go to the next step in the learning process, corresponding to Figure 24.

Although it is mentioned a scoring system for the questionnaires as part of the gamification elements, it is not yet implemented and hopeful it will be available in the next version.

The next screen (Figure 24) doesn't contribute to the selection of the model. However, it contains some basic knowledge in biotechnology with a questionnaire of basic concepts, such as what is fermentation. Furthermore, based on request in the first user experience (Chapter 8) extra information about bioprocesses is also introduced and which can be seen in Appendix 4.1.

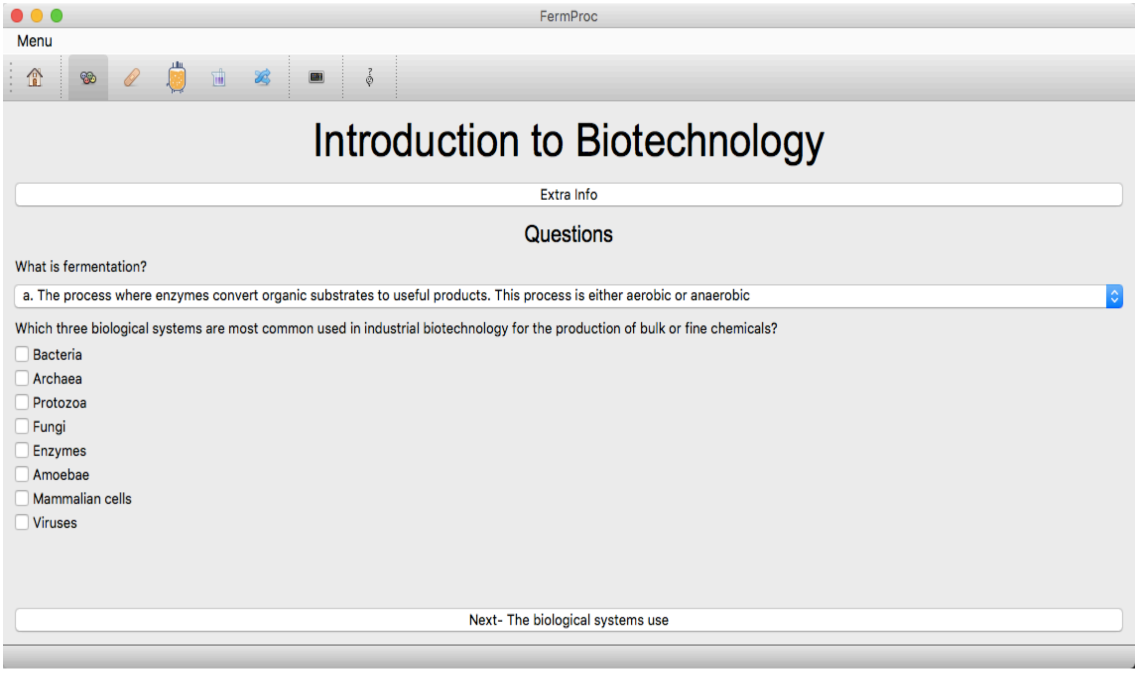


Figure 25. Capture of the screen for the introduction to biotechnology.

From the introduction to biotechnology (Figure 24) it can be accessed to the next learning content, corresponding to the biological system used (Figure 25). In this screen, the microorganisms or biological system are selected based on the biological systems available.

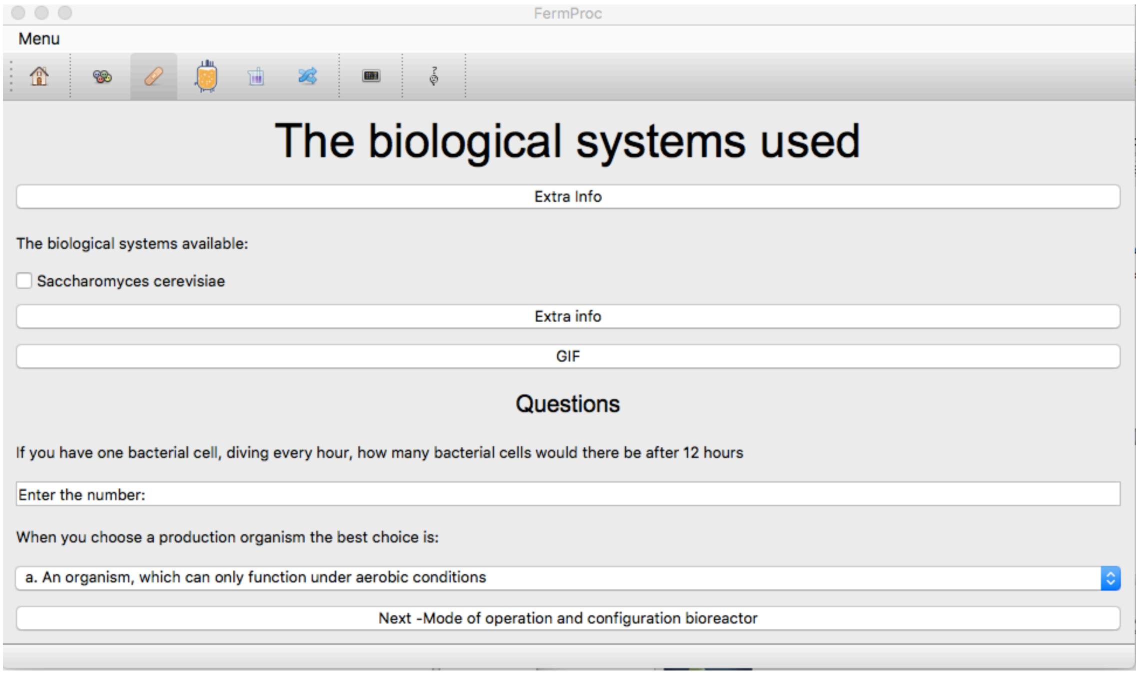


Figure 26. Capture of the screen for the biological system used.

Currently, the only possibility is *Saccharomyces cerevisiae*. Furthermore, based on the petitions of more extra-hints of the learning experience in Chapter 8, it is available extra information about *Saccharomyces cerevisiae* (Figure 26).

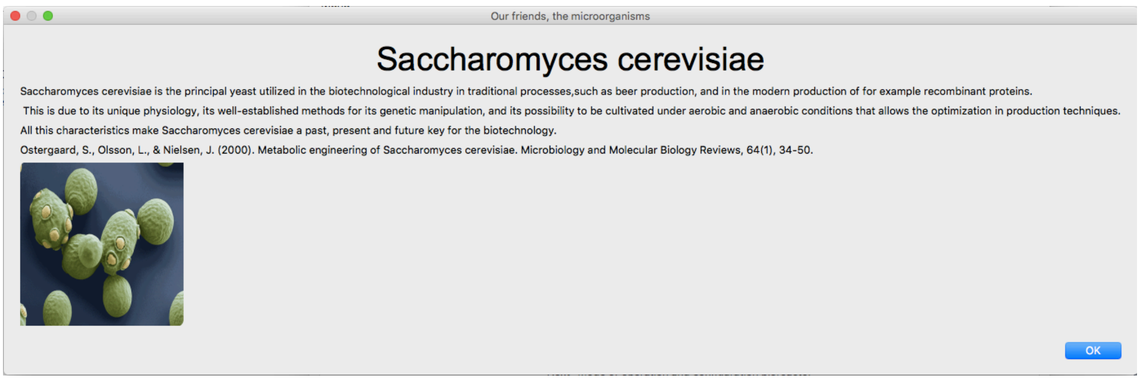


Figure 27. Learning hint about *Saccharomyces cerevisiae.* The information display can be read in Appendix 4.2

It is important to highlight that all the extra-hint and information contains the references so the users can trust the information and avoid mental defenses (mentioned in Chapter 4.2).

- And a gif of the microorganisms.

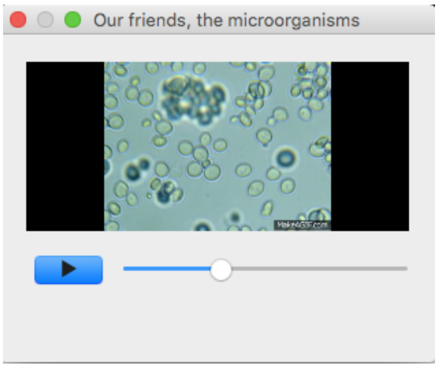


Figure 28. Capture of an implemented GIF of microorganism

The next screen corresponds to the mode of operation and configuration in Figure 28.

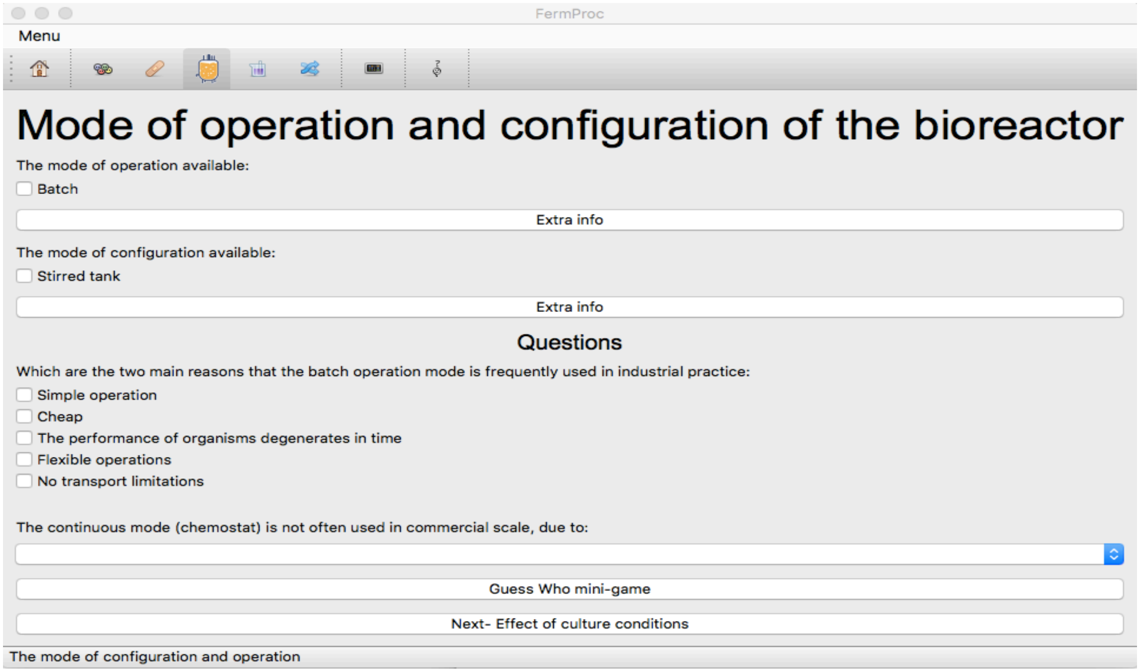


Figure 29. Capture of the screen of "Mode of operation and configuration of the bioreactor".

Figure 28 follows the same "model" that the one for the choosing the biological systems, and the user needs to choose the mode of operation (batch) and the mode of configuration (stirred tank). Both of the choices have the possibility of display more information (Appendix 4.3). Furthermore, in this screen, it is implemented an option to access to a "mini-game" of Guess Who??- Bioreactor version (Figure 30) and which set of rules and explanation can be seen in Figure 29.

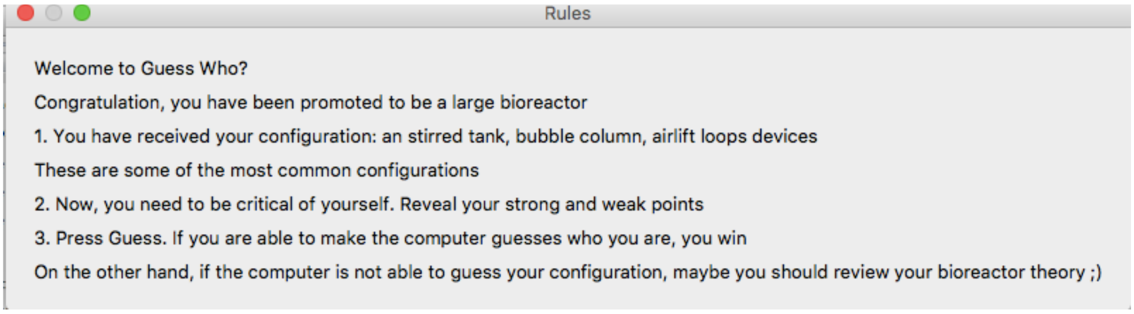


Figure 30. Capture of the rules of the Guess Who? mini-game.

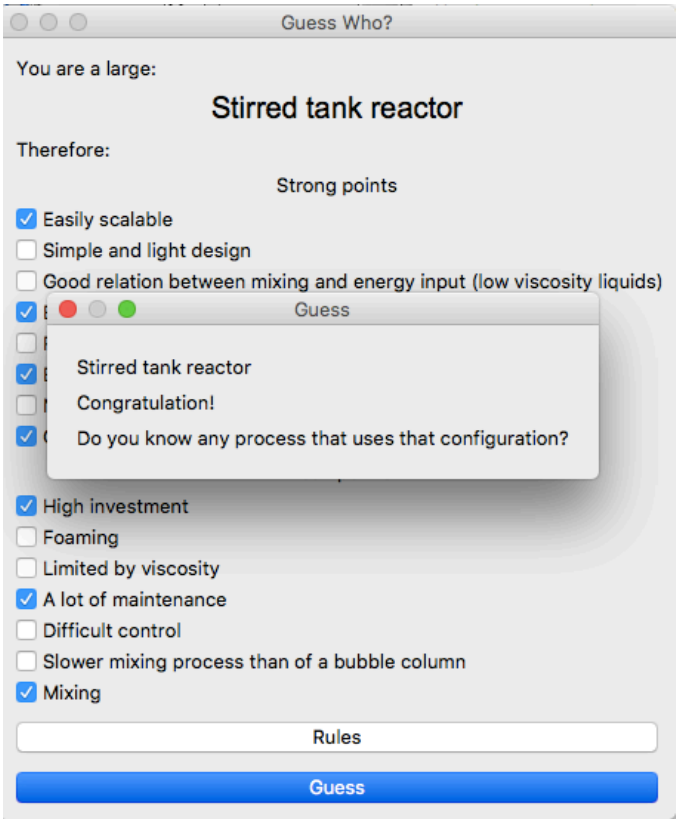


Figure 31. Capture of the Guess Who? mini game.

The next screen corresponds to the effect of culture conditions (Figure 30). This screen requires from the previous selection of the microorganisms (Figure 25), as optimal conditions depends on the biological system. Moreover, the checking of the conditions is part of the learning process as this conditions influence the kinetic model of *Saccharomyces cerevisiae* in a batch stirred tank. Due to the learning objective of the platform, FermProc will ask the user to choose. However, the limited timing didn't allow the creation of different culture conditions. Other possibilities are briefly explained in appendix 4.4.

As an extra-hint, with the choice of glucose and aerobic conditions, it is possible to learn more about "Crabtree effect" (deeply explained in Appendix 4.4).

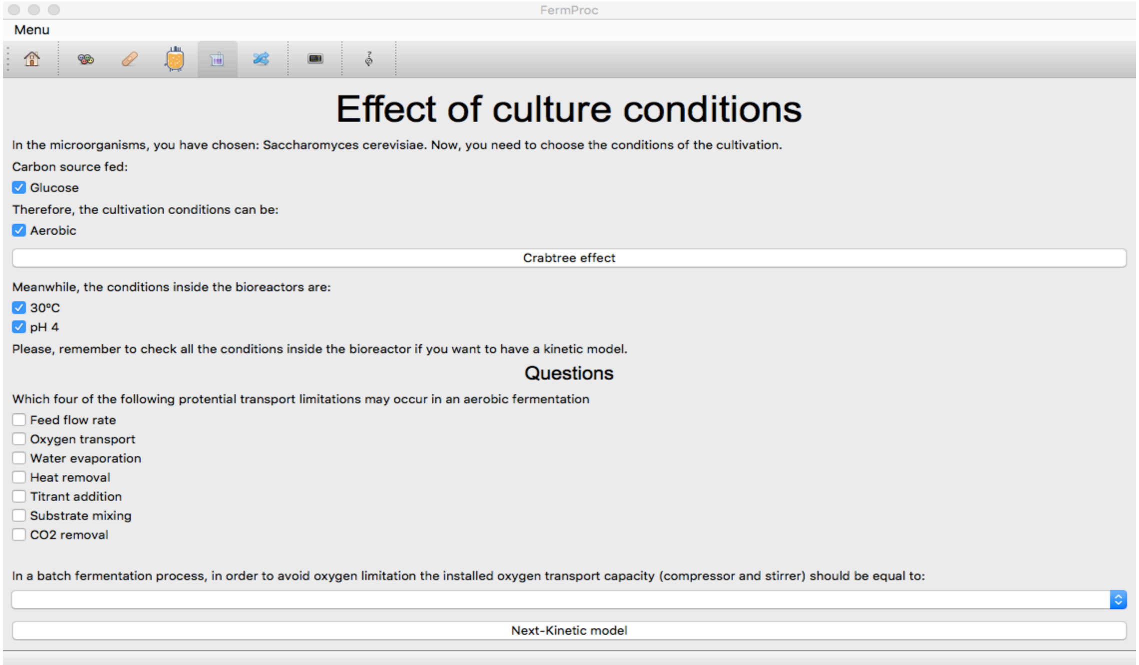


Figure 32. Capture of the screen of "Effect of culture conditions".

Once all the conditions have been checked or if the model template has been previously defined in the initial screen (Figure 22), it is possible to enter in the screen for the simulation of the kinetic model in Figure 32.

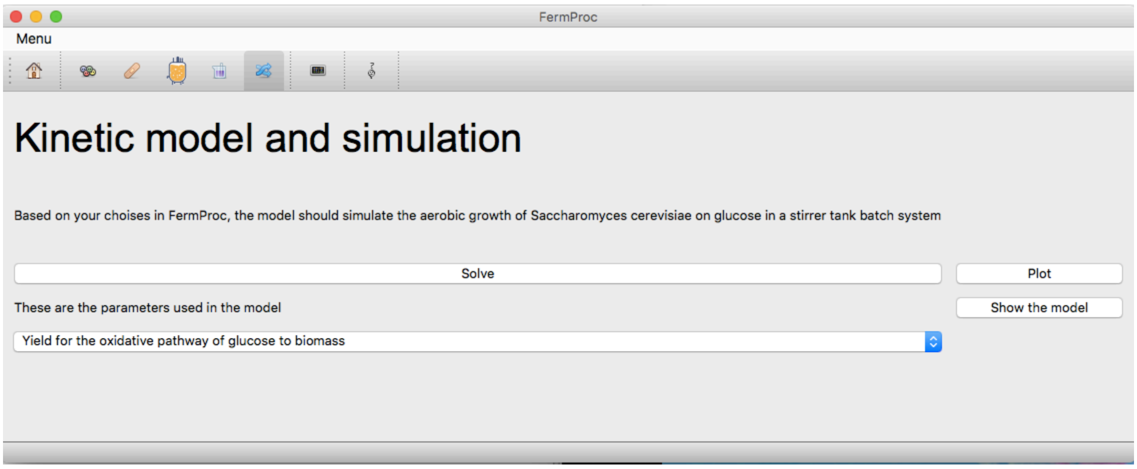


Figure 33. Capture of the screen of "Kinetic model and simulation".

In Figure 32, the process choices (microorganisms, mode of configuration and operation, and cultivation conditions) are automatically imported and the kinetic model object is exported. Furthermore, three buttons are implemented:

- Solve: for the solution of the kinetic model.
- Plot: for the plotting of the concentration of biomass, glucose, ethanol, and oxygen versus time in Figure 32. The early state of the platform makes the graph lacking from axis and legends but these problems will be addressed in the next version of the platform

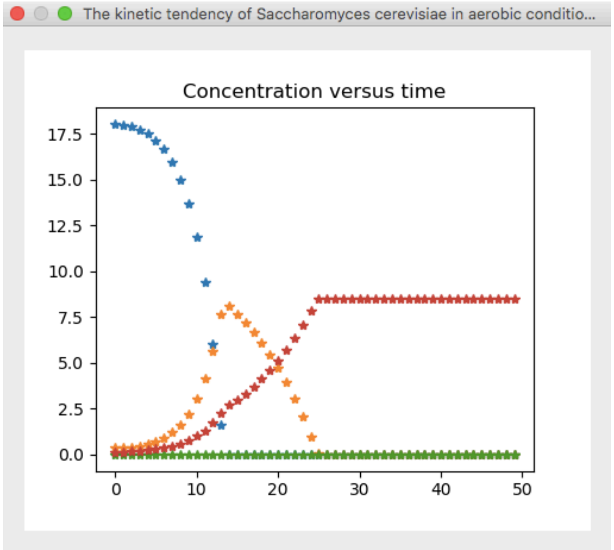


Figure 34. Capture of the window in which it is displayed the output of the kinetic model. The colour code is blue for the concentration of glucose, orange for the concentration of ethanol and red for the concentration of biomass. Y-axis corresponds to the concentration (g/L) while the X-axis is the time (h).

– Show the model: An explicative video of the kinetic model was created. In this video, the overflow that *Saccharomyces cerevisiae* suffers in the cultivations conditions of the case study is explained.

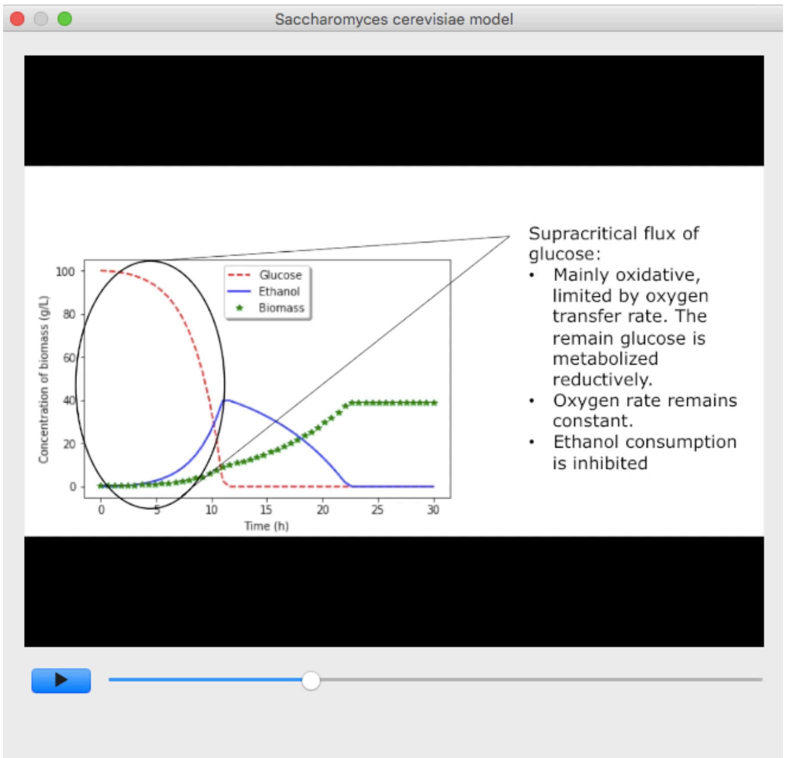


Figure 35. Capture of the video in which the kinetic model implemented is explained.

Furthermore, from Figure 32, it is possible to modify all the parameters in the model collected in Table 4. For example, if the yield for the reductive pathway of glucose to biomass (g biomass/g glucose) is selected, Figure 34 will appear.

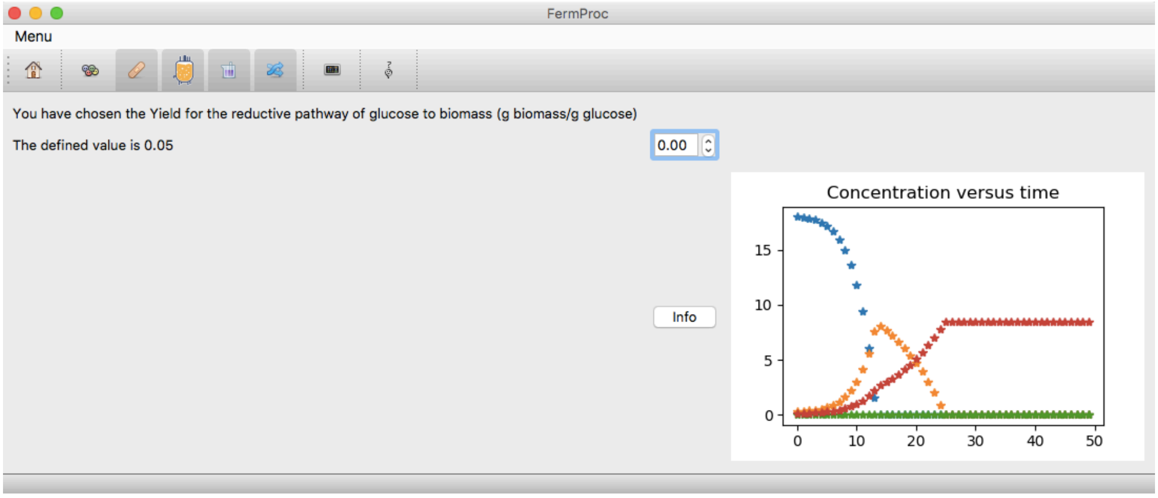


Figure 36. First screen for the modification of the kinetic model. In this example the yield for the reductive pathway of glucose to biomass is choisen. The colour code is blue for the concentration of glucose, orange for the concentration of ethanol and red for the concentration of biomass. Y-axis corresponds to the concentration (g/L) while the X-axis is the time (h).

Then, it is possible to change the value of the parameter and if the value of the yield changes from 0.05 g biomass/g of glucose to 2.0 g biomass/g of glucose and the result is shown in Figure 36.

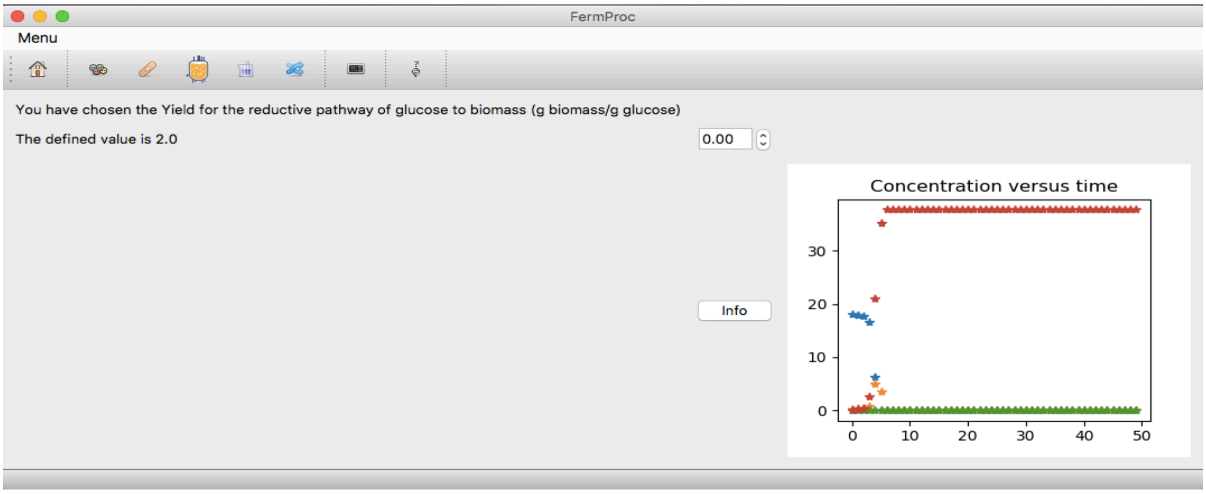


Figure 37. Screen for the modification of the kinetic model, after a variation of the yield value. The colour code is blue for the concentration of glucose, orange for the concentration of ethanol and red for the concentration of biomass. Y-axis corresponds to the concentration (g/L) while the X-axis is the time (h).

Furthermore, in Figure 37, it is possible to see how the consumption of glucose (red) and the production and consumption of ethanol (blue) changed:

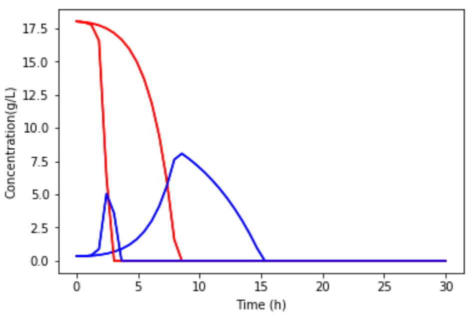


Figure 38. Rate of the glucose (red) and ethanol (blue) modifying the yield of the reductive pathway of glucose to biomass from 0.05 g biomass/g of glucose to 2 g biomass/g of glucose. Due to the still in development knowledge of Python (with which it is generated this graph), it was not possible to make equals the colour code.

Also, it is possible to get information about the parameter by the button "Info" as can be seen in Figure 38.

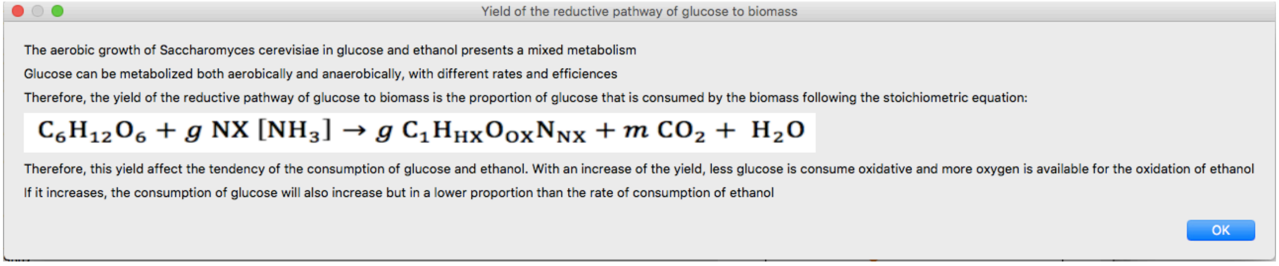


Figure 39. Learning hint for the selected parameter.

The information for the different parameters is collected in the in Appendix 4.5.

Finally and optionally, the toolbar has two more icons.

- . As it has been previously mentioned, it accesses to a series of educational videos. So far, it is available two videos: for the preparation of medium and the dissembling of a 2L fermenter.
- . It is a first draft of a system for the collection of the opinions of the users about the software and to facilitate the future user experiences. It can be seen in Figure 39. This screen is still in development and its implementation is not still integrated in the software architecture

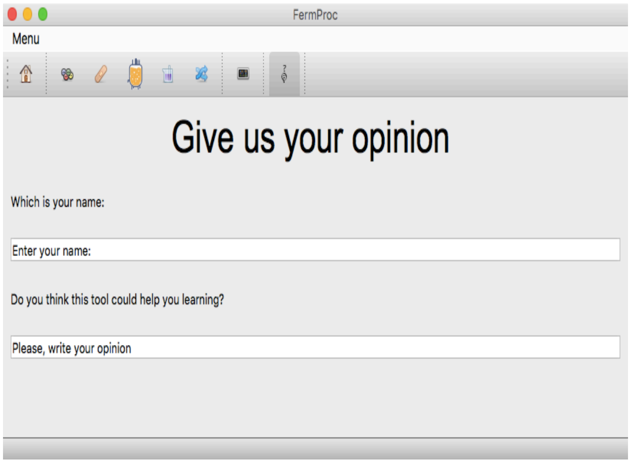


Figure 40. Capture of the screen of "Give us your opinion".

9.3 Conclusions

In this chapter, a quick-look at the software developed for the implementation of the case study (FermProc) was presented. More specifically, FermProc was developed in Python as it allows a rapid and efficient prototyping, whereas as the resulting process simulator of the case study, FermProc requires of an organized and free-flowing learning experience, the possibility of the modification of the model and a variable intrinsic feedback. Furthermore, the resulting software architecture and its implementation will require of a second learning experience for its validation in the nearby future.

10 Conclusions and future work

10.1 Conclusions

In this thesis, a computer-aided framework for the integration of models, and a pedagogical approach with the use of game elements as learning tools has been proposed and tested in a case study. Simulators and gamification have been proven as an effective learning tool, although in the majority of the cases; the commercial simulators used in engineering education lack of an educational objective, and the application of game elements is still a new concept for technical university education. Consequently, and due to the novelty of the proposal of developing a process simulator with a thoughtful learning design and game elements, firstly, a methodology for the creation of such platforms is needed.

Therefore, the achievements in this work are summarized as:

i. A systematic computer-aided framework for the development of pedagogical process simulators using gamification elements is generated and its applicability is demonstrated through a fermentation case study of the aerobic growth of *Saccharomyces cerevisiae* on glucose and ethanol in a batch system. Every step of the framework is clearly explained and its application to other process simulations should be favorable.

ii. The validation of the case study of the computer-aided framework was done through a learning experience, in which 100% of the student participants found the platform developed useful for the learning and asked for an increased of the content. During this experience, it was also created a collaboration frame between the future users and the developer with a constructive feedback for the next steps of the platform.

iii. Considering the information collected during the learning experience, the methodology was implemented in an original software, which was called FermProc. FermProc is a cross-platform desktop application created in Python and PyQt toolkit. It has a software architecture that includes an interactive information flow for the display of learning hints, questionnaires, and the modification the model as well as integrates the creation of a template model library. Furthermore, FermProc provides predefined intrinsic feedback and the possibility of trail different kinetic scenarios, along with hints and theory behind every choice of the user, multimedia content, and a mini-game that were also implemented.

10.2 Future work

Due to the extension of this project and the limited time, some possibilities for future work had been mentioned along this report. However, it is important to highlight that the main future work is the application of the framework to other unit operation, such the downstream process, for a further validation of the computer-aided framework proposed.

Moreover, the future work is deeply explained in this section, along with some suggestions.

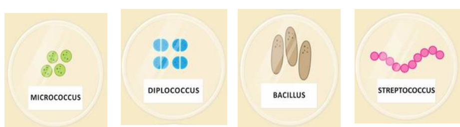
1. *A second learning experience*. The second learning experience is needed due to several modifications that have been implemented after the learning experience, such as in increase in the level of the learning content and the addition of more hints and activities from the first learning experience. On the other hand, the FermProc was briefly presented in two seminars with experts in fermentation and the feedbacks were very positive.
2. *A further validation of the methodology*. The aim of this project has been the development of a methodology. However, the success of the learning experience (in Chapter 8) is not enough for a complete validation of the methodology. And consequently, it will be needed to apply the framework to different process simulators. Furthermore, this creates an excellent opportunity to apply the framework to different operations inside the same platform and provide the students with, apart from the possibility of learning about other operation units, a higher understanding of how the whole process works and is affected.
3. *Improvement of the FermProc*. FermProc is an original graphical user interface and it is still in development. Therefore, it has several areas of improvement, for example:
   - **Increase the complexity of the model.** As it has been previously mentioned, in Chapter 7.3, the balance material of energy and momentum has not been included in the mathematical process description. Furthermore, it was not included physic-chemical equations that simulate the acid-base system or gas-liquid exchange processes of dissolved CO_2, and only a mode of operation (batch) has been implemented. In the near future, it is expected to included the mixed weak acid/base kinetic model of Musvoto et al (1997) [81], which includes all the significant compounds that are consumed or produced in relevant concentrations to affect the pH. And it will also be integrated the gas-liquid exchange process for carbon dioxide, nitrogen, and ammonia. Those considerations will be available to be selected by the user in the graphical user interface and therefore, its application will develop into a reflective observation, abstract conceptualization and a further active experimentation inside FermProc.
   - **Evolution from constructive learning to collaborative learning.** As it was mentioned in Chapter 4, collaborative learning can provide the student with a cooperative environment that favors the exchange of information between peers and could increase the intrinsic feedback. Therefore, it could be interesting to add a chat or a section of the platform in which user could share information or knowledge.

And here, some suggestions and ideas that couldn't been implemented due to the lack of time:

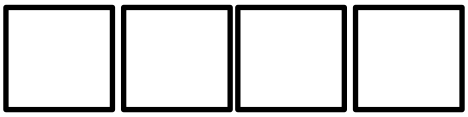
- **Mini-games.**
  1. <u>Flip the card with microorganisms.</u> As different microorganisms have different very distinctive morphologies, it is possible to create a game for the connection and train the memory.

     For example:

     1st: 4 pictures/cards with microorganisms and its names are displayed.

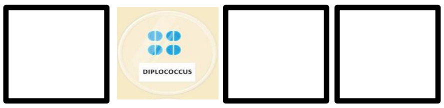


2nd: The cards are flipped



3rd: FermProc is asking you for a specific picture, for example diplococcus

4th: User needs to click the image that corresponds to diplococcus. In this example:



Once the 4 cards have been correctly flipped, the next level will increase the number of cards in the game.

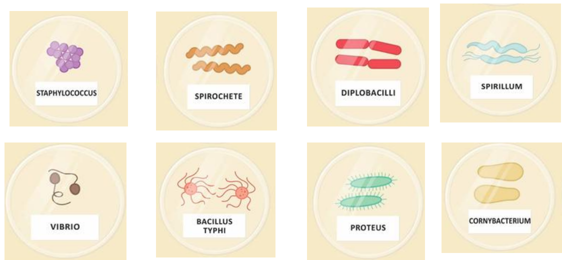


Figure 41. Schematic drawing of different microbial morphologies, drawn by Zhaolifang ©

1. <u>Control your own fermentation</u>. Fermentation is a complex process that is affected by several parameters. Therefore, a possible activity inside FermProc could be the simulation of a complete fermentation with its control system. The challenge will be a series of "problems" sent by the programme such as: *OOHH, No. Maybe an Escherichia coli has entered in the bioreactor for the aerobic growth of S.cerevisiae. What can we do?* Therefore, a possibility for the student is dropping the pH at 4. This pH is the optimal for *S.cerevisiae* but *Escherichia coli* is neutrophile and prefers environments with near-neutral pH [82]. This is a simplified solution that doesn't cover the complexity of the contamination of a pure-culture fermentation.
2. <u>Puzzles.</u> Once it is increased the number of operations available, the user can organized units operation in a logical way, in order to arrive to the "perfect" process design.

These are only some of the possibilities. As FermProc is created from scratch, it doesn't have any design constraint and the limit is only in our abilities and imagination.

Glossary

Term	Definition
Model	The representation of a real or virtual physic-chemical, economic, social or human situation, in an alternate mathematical or physical form, for an envisaged purpose [83], [84].
Modeling methodology	Methodology for the process of model development and application, that is, representation of the modeling process in terms of an ordered set of tasks and sub-tasks. In this work, a methodology based on workflows and data-flows for the different sub-tasks of the modeling process is proposed[83], [84].
Workflow	A workflow summarizes the different steps required to complete a given task. In this context 'in-depth' provides a detailed explanation of each work-flow step and the corresponding sub-steps[83], [84].
Data-flow	Required data/information for different workflow (sub-) steps as well as output data/information[83], [84].
Computer-aided modeling framework	A computer-aided modeling framework provides the architecture through which the computer-aided methods and tools can be implemented and used according to the work-flow and data-flow of the methodology [83], [84].
Modeling tool/software	Actual implementation of computer-aided modeling framework in a software[83], [84].
Paper prototype	Hand drawing of user interface in order to enable a rapid design, simulation and test.
Learning design	A learning design describes a sequence of learning activities that learners undertake to attain some learning objectives, including the resources and support mechanisms required. It is conditioned by the target group, a specific context and/or a knowledge domain[85], [86].
Gamification	It is the use of game design elements in non-game contexts [47].
Fermentation	The conversion of organic substances by microorganisms. In the classical definition, this process is anaerobic but the definition also has a wider used, including aerobic processes.
Software architecture	It is a depiction of a program or computing system that aids in understanding how the system will behave [87].

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Appendix 1

```
#!/usr/bin/env python3
# -*- coding: utf-8 -*-
"""
Created on Mon Jan 29 09:15:34 2018

@author: tsukuru
"""

from scipy.integrate import odeint
#Package for plotting
import matplotlib.pyplot as plt
#Package for the use of mathematical expressions
import math
#Package for the use of vectors and matrix
import numpy as np

#Yield
Yox_XG = 0.8
Yred_XG = 0.05
Yox_XE = 0.72
Y_OG = 1.067
Y_EG = 0.5
Y_OE = 1.5
#Biological parameters
q_g = 3.5
q_o = 0.37
q_e = 0.32
t_lag = 4.66
Kg = 0.17
Ko = 0.0001
Ke = 0.56
Ki = 0.31
O_sat = 0.00755
kla = 1004


#number of processes
n = 4
#number of components
m = 4
#initialize the stoichiometric matrix, s
s = np.zeros((m,n))

s[0,0] = -1
s[0,1] = 0
s[0,2] = -Y_OG
s[0,3] = Yox_XG

s[1,0] = -1
s[1,1] = Y_EG
s[1,2] = 0
s[1,3] = Yred_XG

s[2,0] = 0
s[2,1] = -1
```

```
s[2,2] = -Y_OE
s[2,3] = Yox_XE

s[3,0] = 0
s[3,1] = 0
s[3,2] = 1
s[3,3] = 0

#time
t = np.linspace(0, 30)
#initialize the rate vector
rho = np.zeros((4,1))
##initialize the overall conversion vector
r=np.zeros((4,1))
#initial concentration vector
G0 = 18
E0 = 0.34
O0 = 0.00755
X0 = 0.1
C0 = np.array([G0, E0, O0, X0])
#function to calculate the rate
def rxn(C,t):

    rho[0,0] =
(1/Y_OG)*min(q_o*(C[2]/(C[2]+Ko)),Y_OG*(q_g*(C[0]/(C[0]+Kg))))
    rho[1,0] = (1-math.exp(-t/t_lag))*((q_g*(C[0]/(C[0]+Kg)))-
(1/Y_OG)*min(q_o*(C[2]/(C[2]+Ko)),Y_OG*(q_g*(C[0]/(C[0]+Kg)))))
    rho[2,0] = (1/Y_OE)*min(q_o*(C[2]/(C[2]+Ko))-
(1/Y_OG)*min(q_o*(C[2]/(C[2]+Ko)),Y_OG*(q_g*(C[0]/(C[0]+Kg)))),Y_OE*(q
_e*(C[1]/(C[1]+Ke))*(Ki/(C[0]+Ki))))
    rho[3,0] = kla*(O_sat - C[2])

    #Developing the matrix, the overall conversion rate is
stoichiometric *rates
    r[0,0] =
((s[0,0]*rho[0,0])+(s[1,0]*rho[1,0])+(s[2,0]*rho[2,0])+(s[3,0]*rho[3,0
]))*C[3]
    r[1,0] =
((s[0,1]*rho[0,0])+(s[1,1]*rho[1,0])+(s[2,1]*rho[2,0])+(s[3,1]*rho[3,0
]))*C[3]
    r[2,0] = ((s[0,2]*rho[0,0])+(s[1,2]*rho[1,0])+(s[2,2]*rho[2,0]))
*C[3]+(s[3,2]*rho[3,0])
    r[3,0] =
(s[0,3]*rho[0,0])+(s[1,3]*rho[1,0])+(s[2,3]*rho[2,0])+(s[3,3]*rho[3,0]
) *C[3]

    #Solving the mass balances
    dGdt = r[0,0]
    dEdt = r[1,0]
    dOdt = r[2,0]
    dXdt = r[3,0]
    return [dGdt,dEdt,dOdt,dXdt]

C = odeint(rxn, C0, t, rtol = 1e-7, mxstep= 500000)

print (C)
plt.plot(t, C[:, 0],'r', label='Glucose theoretical')
plt.xlabel('Time (h)')
```

```
plt.ylabel('Concentration of glucose (g/L)')
plt.plot(t, C[:, 1],'b',label='Ethanol theoretical')
plt.xlabel('Time (h)')
plt.ylabel('Concentration of ethanol (g/L)')
plt.plot(t, C[:, 3],'g', label='Biomass theoretical')
plt.xlabel('Time (h)')
plt.ylabel('Concentration of biomass (g/L)')
plt.legend(loc='upper center', shadow=True)
plt.xlim([0, 30])
plt.ylim([0, 20])
```

Appendix 2

This appendix contains the experimental setting for the aerobic cultivation of *Saccharomyces cerevisiae* CEN.PK 113-7D in glucose in a batch in a laboratory.

A2. 1. Medium preparation

Table A2.1. Medium recipe

Compound	Amount
$(NH_4)_2SO_4$	2 g/L
$K_2HPO_4 \cdot 3H_2O$	2 g/L
$MgSO_4 \cdot 7\,H_2O$	0.5 g/L
KCl	2 g/L
Yeast extract	11 g/L
Glucose $\cdot H_2O$	11 g/L
H_2SO_4	5 mol/L
NaOH	(Adjust pH to 4.5)
PEG Antifoam	1 mL in 2 L bioreactor

Note: Transfer into the prepared culture vessel and autoclave at 121ºC for 20 minutes. It is important to highlight that glucose needs to be sterilized separately from the rest of the medium.

A2.2 Assembling the bioreactor

- Calibration and installation of the pH-electrode.
- Installation of the pO_2 probe.
- Preparation and sterilization of base, acid and antifoam.
- Sterilization of the culture vessel including the medium.
- Calibration of the pO_2 probe at cultivation mixing speed.
- Sterilize connection of peripheral equipment.

A2.3 Culture conditions

- Culture volume: 2L

- Temperature: 30ºC
- pO_2: 40ºC, controlled
- pH value: 4.5, controlled
- Stirrer: 250 rpm
- Aeration: 1 vvm
- The initial concentration of microorganisms is approximately 0.1g/L

A2. 4 Analytical procedures

Biomass will be measured by optical density with the spectrophotometer at wavelength of 600mm and the dilution should be to get a measured extinction between 0.2 and 0.4.

On the other hand, glucose and ethanol can be measured by the HPLC.

Table A2.2. Experimental data obtained in a fermentation

Time (h)	Glucose (g/L)	Ethanol (g/L)
0	18.18	0.348
0.25	18.19	0.399
0.5	18.13	0.428
0.75	17.98	0.479
1	17.01	0.473
1.5	17.05	0.553
1.75	17.09	0.554
2	17.40	0.672
2.25	17.19	0.788
2.75	17.07	0.838
3	16.84	0.886

Time (h)	Glucose (g/L)	Ethanol (g/L)
3.25	16.27	1.15
3.75	15.05	1.30
4.25	14.72	1.65
5.25	12.79	0.672
6.25	9.67	2.46
8.28	0	3.68
10.28	0	7.63
12.28	0	2.36
14.28	0	1.14
16.28	0	0.207

Appendix 3

Section for Sustainable Biotechnology
Department of Chemistry
and Bioscience Aalborg
University Copenhagen

Questionnaire for the test of the paper prototype 29/11/2017

Please, qualified the questions from totally agree (2), agree (1), neutral (0), disagree (-1) or totally disagree (-2).

1. Do you think this tool could help you learning about a specific fermentation?
   ....

   Could you explain with your own words how the tool helps/doesn't help you in learning about fermentation? Please give one or two examples...

2. Do you like the idea of gamification (using some things from games) for studying?
   ....

3. Do you think you could extrapolate the knowledge to a "hand-on" process?
   ....

   Could you explain with your own words how the tool helps/doesn't help you in an extrapolation of theory into actual fermentation? Please give one or two examples...

4. Do you consider the content easy?
   ....

   If you agree or strongly agree, could you help us to increase the level?

5. Do you like the feedback?
   ....

   (for example; Congratulation, you are rocking it) Please give one or two examples of why do you like it or not...

6. Do you like the interface designed?
....

Please give one or two examples of why do you like it or not...

As we believe in co-design, please, how can we improve the game? (Evaluate in a 100 % scale)

(a) Make the game more challenging

(b) Improve the game storyline

(c) Add more tasks

(d) Clarify the game objective

(e) Provide more information before playing.

(f) Make the interface more attractive.

And finally, do you have any thoughts on have to improve this software?

We are very grateful about your feedback

Appendix 4

In this appendix, it is embedded the theory implemented in FermProc. More specifically, the theory collected here, covers the learning content previously mentioned in the learning design (Table 5) of the case study of the methodology proposed in this thesis. It is important to highlight that in FermProc and in this appendix, it is only included a tiny part of the available theory of fermentation process, and in the future more content will be included in FermProc. In fact, the theory presented is highly dependent on the modeling assumptions (Chapter 7) and tries to be an understandable overview of the related process for bachelor level students. For example, the choice of *Saccharomyces cerevisiae,* aerobic cultivation, and glucose as fed carbon source leads to the Crabtree Effect (in Appendix 4.4) but the inside of the metabolic routes are not included and will be further explained in the next version of the platform.

Consequently, FermProc specifically covers the theory related to the aerobic growth of *Saccharomyces cerevisiae* in glucose and ethanol and its menu includes the areas of:

- Introduction to biotechnology.
- Microorganisms.
- The configuration of the bioreactor.
- Culture conditions.
- Kinetic model and simulation.

A4.1 Introduction to biotechnology

Bioprocesses are an essential part of food, chemical, and pharmaceutical industries; and industrial fermentation is applied for the production of a wide range of industrial products. Further than the design of all the fermentation requirements; economical, and environmental and safety assessments needs to be done. However, in this platform (FermProc), they won't be addressed but more information can be found in literature.

Bioprocesses are characterized by the complex relationship among their steps [88]. Those are:

1. Development of the inoculum. It is the amount of microorganism(s) that will enter in the medium. It can be pure or modified. Commonly, the maximum biomass in the minimum time is aimed.
2. Selection of the medium. It is done in 3 steps:
    i. Design.
    ii. Formulation.
    iii. Optimization.
3. Sterilization of the medium. The principal technique will be the autoclave but other possibilities, such as sterilized filtration, are available.
4. Fermentation. The bulk growth of microorganisms on a growth medium.
5. Cellular separation. It involves the separation of the biomass from the rest of the products. It is commonly done by filtration and/or precipitation.
6. Product separation. It is based on the different properties that the compounds present.
7. Purification of the products.

Yet, biotechnology is the use of biological systems to develop or make a valuable product and the correct choice of the biological system will be the "first" step of the process.

More information can be found, for example in:

- P. M. Doran, *Bioprocess Engineering Principles*. 1995.

A4.2 Biological system

A biological system can be defined as microorganisms or their derivatives, such as enzymes. Some of the microorganisms and the products associated can be seen in Table A4.1.

Table A4.1 A brief overview of fermentation products, and an example of its host organism. It is based on [89]

Product	Producing strain
Alcohols	
Ethanol	*Saccharomyces cerevisiae*
Butanol/Acetone	*Clostridium acetobutylicum*
Organic acids	
Citric acid/Gluconic acid	*Aspergillus niger*
Lactic acid	*Lactobacillus delbrueckii*
Amino acids	
L-glutamic acid	*Corynebacterium glutamicum*
L-lysine/ L-arginine	*Brevibacterium flavum*
Antibiotics	
Penicillins	*Penicillium chrysogenum*
Tetracycline	*Strepotomyces auerofaciens*
Enzymes	
Cellulase	*Trichoderma reesei*
Protease	*Bacillus licheniformis*
Pectinase	*Aspergillus niger*
Polymers	
Dextran	*Leuconostoc mesenteroides*
Xanthan	*Xanthomonas campestris*

Saccharomyces cerevisiae

Saccharomyces cerevisiae is the principal yeast utilized in the biotechnological industry in traditional processes, such as beer production, and in the modern production of for example recombinant proteins [69]. This is due to its unique physiology, its well-established methods for its genetic manipulation, and its possibility to be cultivated under aerobic and anaerobic conditions that allows the optimization in production techniques. Also, due to its long history of safely use and consumption, and lack of production of toxins, most strains of *S. cerevisiae* have generally been considered as safe (GRAS). All these characteristics make *Saccharomyces cerevisiae* a past, present and future key for biotechnology [90].

More information of the choice of the microorganisms:

- P. M. Doran, *Bioprocess Engineering Principles*. 1995.

More information of the *Saccharomyces cerevisiae:*

- E. A. Johnson and C. Echavarri-Erasun, *Yeast biotechnology*, vol. 1. Elsevier B.V., 2011.

A4.3 Mode of operation and configuration of the bioreactor

Although a fermenter, or bioreactor, is any device or vessel used in the bioreaction, its design is a complex task. Bioreactors differ from chemical reactors, as the support and the control of biological entities require a higher degree of control over process upsets and contamination due to the sensitivity and less stability of biological systems. The design of fermentation requires of scientific and engineering knowledge and relies on many rules of thumb [27], [91]. Therefore, there is not a universal bioreactor and the size can vary from a few mm^3, in laboratory scale, to 500 m^3. However, a train of bioreactors ranging from 20 liters to 250 m^3 [27] is more typically configuration found in a bioproduction facility.

The main factors for the design of a bioreactor are [91]:

i. Reactor configuration. How should the vessel be in order to provide an adequate mixing, aeration, etc. in a specific process?
ii. Reactor size. What is the required size in order to achieve the desired production rate?
iii. Process conditions inside the reactor. What are the optimal reactions conditions for the specific process? The main key factors will be:
    a. Agitation rate.
    b. Oxygen transfer.
    c. pH.
    d. Temperature.
    e. Foam production.
iv. Mode of operation. How should the introduction and exit of substrates and products be?

Due to the lack of time in this project was only implemented batch as the mode of operation and stirred tank as reactor configuration.

Batch

It is the simplest mode of operation. Once the fermentation has started, there is no additional inflow of substrate and nutrients and no outflow of fermentation broth and, if there is no leaks or evaporation from the vessel, the liquid volume in batch reactors can be considered constant.

The typical batch microbial growth curve has the following phases [91]:

1. Lag phase. Cells need to adapt to the new environment; and therefore there is no or very little growth.
2. Acceleration phase. Growth starts.
3. Growth phase. Growth achieves its maximum rate.
4. Decline phase. Growth slows due to nutrient exhaustion or build-up of inhibitory products.
5. Stationary phase. Growth ceases.
6. Death phase. Cells lose viability and lyse.

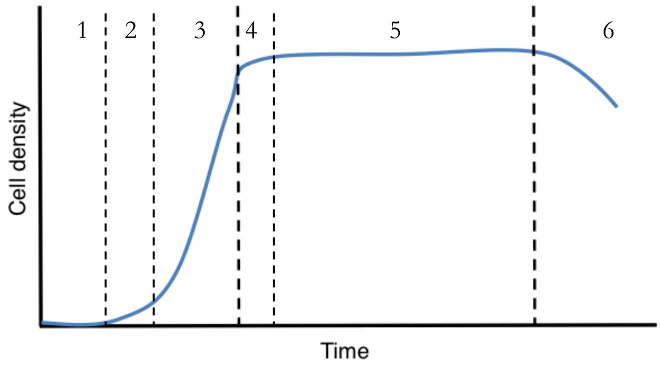

Figure A4.1. Curve of the cell density versus time in a typical batch growth

Overall, batch bioreaction systems have a number of advantages and disadvantages [92] that are collected in Table A4.2:

Table A4.2. Brief overview of the advantages and disadvantages of batch as mode of configuration

Advantages	Disadvantages
Reduced risk of contamination or cell mutation, due to a relatively brief growth period.	Lower productivity levels due to time for filling, heating, sterilizing, cooling, emptying and cleaning
Lower capital investment when compared to continuous processes for the same bioreactor volume	Increased focus in instrumentation due to frequent sterilization
More flexibility with varying product/biological systems.	Higher cost for labor and/or process control for this non-stationary process.
Higher raw material conversion levels, resulting from a controlled growth period.	Larger industrial hygiene risks due to potential contact with pathogenic microorganisms or toxins

Furthermore, common applications for batch bioreactors include:

- Products that must be produced with minimal risk of contamination or organism mutation.
- Operations in which only small amounts of product are produced.
- Processes using one reactor to make various products.
- Processes in which batch or semi-continuous product separation is adequate.

More information can be found in:

- J. A. Williams, "Keys to bioreactor selections," *Chem. Eng. Prog.*, vol. 98, no. 3, pp. 34–41, 2002.

Stirred-tank

Stirred-tank reactors have been widely implemented for biological applications. Its operation principles are relatively simple, it is characterized by a stirring mechanism inside the tank and the growth is suspended or immobilized by carrier. For optimal mixing, the tank features not only an agitator system but also baffles. As the bioreaction progresses, the bubbles, produced by the air supply, are broken up by agitators and they travel upward. It is the most common reactor in the industry up to volumes of 22 m^3[27]. The height-to-diameter ratio of the vessel can vary, depending on heat removal requirements.

More information can be found in:

- M. Chisti, Yusuf and Moo-Young, "Bioreactor Design," *Biotechnol. C. Kristiansen, B. eds). Cambridge Univ. Press. Cambridge*, pp. 151–171, 2001.

Guess Who?? (Bioreactor version)

Table A4.3. Characteristics chosen for the Guess-Who bioreactor version mini-game.

Configuration	Strong points	Weak points
Stirred tank	• Easily scalable • Easy control • Easy cleaning • Good gas transfer	• High investment • A lot of maintenance • Mixing
Bubble column	• Simple and light design • No moving parts • Easy cleaning • Good relation between mixing and energy input (low viscosity liquids) • Good gas transfer	• Difficult control • Foaming • Limited by viscosity
Airlift loops devices	• Simple and light design. • No moving parts. • Easy cleaning. • Good gas transfer. • Recirculation loop can be used for cooling.	• Difficult control. • Foaming. • Slower mixing process than of a bubble column.

A4.4 Effect of culture conditions

As it was previously mentioned, some microorganisms can grow in aerobic and anaerobic conditions or use different carbon sources. For example, *Saccharomyces cerevisiae* can use maltose instead of glucose, modifying the kinetic model, as the carbon source needs of a hydrolization by intracellular maltase into two glucose units [93]. Other possibilities are the relation between temperature and rate follows an equation type

Arrhenius, and therefore it should be possible to vary the temperatures, between 25 and 40ºC due to the mesophilic character of the *Saccharomyces cerevisiae,* and see the effect in the kinetic rate.

With the choice of glucose and aerobic cultivation, it is the possible to learn more about "Crabtree effect".

Crabtree effect

Saccharomyces cerevisiae is a crabtree-positive yeast. Therefore, it can use fermentation even in the presence of oxygen, where they could, in principle, rely on the respiration pathway. This phenomenon is observed in most species of the *Saccharomyces, Schizosaccharomyces, Debaryomyces, Brettanomyces, Torulpsis, Nematospora* and *Nadsonia* genera [70]. This is surprising as fermentation has a much lower ATP yield than respiration (2 ATP instead approximately 18 ATP per glucose) [94]. However, this switching mode of metabolism allows a fast energy production in the fermentation, while the respiration maximize the use of energy transformation for ATP production [95].

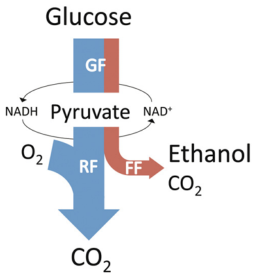

Figure 42. Schematic description of the combination of the respiration and fermentation in the Crabtree effect [96]. It shows that the crabtree-positive yeast posses an upregulated aerobic (blue) and anaerobic (red) glycolytic pathway, even under fully aerobic conditions, when energy and carbon-source is limiting.

Therefore, the selective advantages for this kind of metabolic pathway are the increased rate of ATP production and the toxicity of ethanol that can contribute to the selective advantage compared to only the respiration pathway [94].

For more information, the learners can reach the papers:

- A. Hagman and J. Piškur, "A study on the fundamental mechanism and the evolutionary driving forces behind aerobic fermentation in yeast," *PLoS One*, vol. 10, no. 1, 2015.
- T. Pfeiffer and A. Morley, "An evolutionary perspective on the Crabtree effect," *Front. Mol. Biosci.*, vol. 1, no. October, pp. 1–6, 2014.
- K. Otterstedt *et al.*, "Switching the mode of metabolism in the yeast Saccharomyces cerevisiae," *EMBO Rep.*, vol. 5, no. 5, pp. 532–537, 2004.

A4.5 Kinetic model and simulation

The kinetic models can be used, at least in principle, to increase detailed understanding and to predict and evaluate the effects of adding, removing or modifying molecular components. These relationships are normally expressed in the form of mathematical equations, in which variables include any properties that are of importance for the process [97].

As it was explained previously, under these conditions, *Saccharomyces cerevisiae* presents a Crabtree effect. Therefore, it can proceed to the degradation of glucose via two pathways under conditions of aerobic ethanol formation. Meanwhile, ethanol can be used oxidatively only.

Consequently, in the model, it is presented in two phases:

- The supracritical flux of glucose phase. In this phase, the glucose that cannot be metabolized purely oxidatively is metabolized reductively with accumulation of ethanol [67]. Therefore, the available oxygen governs the kinetic rates. In Table A4.4 the terms of the equations that control the kinetic model are highlighted.

Table A4.4. Kinetic model for the growth of *S.cerevisiae* and its controlled terms in supracritical flux of glucose.

Process	Equation
Oxidation of glucose rate (μ_G^{Oxid})	$\mu_G^{Oxid} = Y_{XG}^{Oxid} \frac{1}{Y_{OG}} \left(\min\left(q_o \cdot \frac{C_{O_2}}{C_{O_2} + K_0} \right), Y_{OG} q_s \frac{C_G}{C_G + K_G} \right)$
Reduction of glucose rate (μ_G^{Red})	$\mu_G^{Red} = Y_{XG}^{Red} (q_G \frac{C_G}{C_G + K_G} - \frac{1}{Y_{OG}} \left(\min\left(q_o \cdot \frac{C_{O_2}}{C_{O_2} + K_0} \right) Y_{OG} q_G \frac{C_G}{C_G + K_G} \right)$
Oxidation of ethanol rate (μ_E)	$\mu_E = Y_{XE} \left(\min\left(q_o \cdot \frac{C_{O_2}}{C_{O_2} + K_0} \right) - \min(k_o \cdot \frac{C_{O_2}}{C_{O_2} + K_0}, Y_{OG} q_G \frac{C_G}{C_G + K_s}), Y_{OE} q_E \frac{C_E}{C_E + K_E} \frac{K_i}{C_G + K_i} \right)$

- The subcritical flux of the glucose phase. . In this phase, the glucose flux is subcritical but there is additional ethanol in the medium and it is utilized oxidative [67]. In Table A4.5, the terms of the equations that control the kinetic model are highlighted.

Table A4.5. Kinetic model for the growth of *S. cerevisiae* and its controlled terms in subcritical flux of glucose.

Process	Equation
Oxidation of glucose rate (μ_G^{Oxid})	$\mu_G^{Oxid} = Y_{XG}^{Oxid} \frac{1}{Y_{OG}} \left(\min\left(q_o \cdot \frac{C_{O_2}}{C_{O_2} + K_0} \right), Y_{OG} q_s \frac{C_G}{C_G + K_G} \right)$
Reduction of glucose rate (μ_G^{Red})	$\mu_G^{Red} = Y_{XG}^{Red} (q_G \frac{C_G}{C_G + K_G} - \frac{1}{Y_{OG}} \left(\min\left(q_o \cdot \frac{C_{O_2}}{C_{O_2} + K_0} \right) Y_{OG} q_G \frac{C_G}{C_G + K_G} \right)$
Oxidation of ethanol rate (μ_E)	$\mu_E = Y_{XE} \left(\min\left(q_o \cdot \frac{C_{O_2}}{C_{O_2} + K_0} \right) - \min(k_o \cdot \frac{C_{O_2}}{C_{O_2} + K_0}, Y_{OG} q_G \frac{C_G}{C_G + K_s}), Y_{OE} q_E \frac{C_E}{C_E + K_E} \frac{K_i}{C_G + K_i} \right)$

More information can be found:

- B. Sonnleitner and O. Käppeli, “Growth of Saccharomyces cerevisiae is controlled by its limited respiratory capacity: Formulation and verification of a hypothesis,” *Biotechnol. Bioeng.*, vol. 28, no. 6, pp. 927–937, 1986.

Furthermore, FermProc has implemented brief information about the parameters of the model that can be modified in the learning platform, including the Table 4 with the units. The short description implemented for each of them are based on the previous work of Doran [91] and M. Maier[98].

- Yield. It specifies the amount of product obtained from the substrate. In the model implemented, the yields are corresponding to:
  - Yield for the oxidative pathway of glucose to biomass.
  - Yield for the reductive pathway of glucose to biomass.
  - Yield of the pathway of ethanol to biomass.

  This three are measurable coefficients that are proportional to the stoichiometric coefficients of production of biomass (in biomass) in the Eq. (1), Eq. (2), and Eq. (3).

  - Yield of the need of oxygen to glucose.
  - Yield of the conversion of glucose in ethanol.
  - Yield of the need of oxygen to ethanol.
- Specific rate. It reflects intrinsic properties of the degrading microorganism, the substrate, and the temperature of growth
  - Of glucose
  - Of oxygen
  - Of ethanol
- Saturation parameter. As the specific rate, it reflects intrinsic properties of the degrading microorganism, the substrate, and the temperature of growth
  - For glucose uptake.
  - For oxygen uptake.
  - For ethanol uptake.
- Inhibition parameter: free glucose inhibits ethanol uptake.
- Lag time. It is the time required for the physiological adaptation of the cells to the new environment. During this period, the growth rate is essentially zero. The lag phase usually lasts from minutes to several hours and it is dependent on the type of medium as well as on the initial inoculum size.
- Concentration of saturated oxygen. It corresponds to the maximum amount of oxygen gas that can be dissolved in the medium. It is highly depended on the overall pressure, the composition of the gas phase and temperature or the composition of the medium (e.g. the presence of salts)
- Mass transfer coefficient for oxygen. It is the product of the resistance coefficient and the total surface of the bubbles present in the fermentation broth. It is dependent on the bubble size and medium composition.