Application for the Technology Development Fund
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Grands for applied research projects

PROJECT DESCRIPTION

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Project title: Biotech Beer Brewing

SUMMARY

1 Project description

Traditional beer brewing is done in a batch process by fermenting glucose to alcohol using Saccharomyces cerevisiae. In a first step wort is produced by mixing water, malt and hop and applying different enzymatical processes. As in a batch process the densities of metabolites in the culture are not controlled the fermentation product and so the quality of the beer is highly dependent on the composition of the wort. To enhance the product quality the process of fermentation must be understood in detail and correlations between the starting conditions and fermentation results must be found. This is typically done in experiments which are very time and cost intensive. Especially high effort is needed to reproduce starting conditions and if different yeast mutants or contamination by other bacteria shall be tested. These experiments are very costly and are not affordable for smaller breweries. A simulation approach to test different starting conditions will reduce the amount of experiments and so the costs and will enable development of new production methods also for smaller companies.

To be able to simulate the fermentation outcome three major requirements must be fulfilled: (1) The starting conditions and dynamic environment constants must be determined, (2) the involved bacteria and internal (enzymatical) processes must be modeled sufficiently and (3) a proper simulation framework must be available which combines all information of (1) and (2) and calculates the fermentation outcome. As the formulation of sufficient models for (1) and (2) depends on the production process, so the applied yeast and wort, this project will concentrate on the development of a simulation framework to enable the simulation of the fermentation products dependent on the fermentation's starting conditions.

To be usable to enhance the fermentation process the simulation framework will enable the access to different process and product parameters. The growth and metabolite production of Saccharomyces cerevisiae in a typical environment including contamination with other bacteria in a batch process will be simulated. The simulation will be parameterised by the used bacteria models and environmental constants which defines the composition and dynamics of the wort (starting conditions). The simulation results are used to formulate quality measures of the fermentation product to finally investigate correlations to the simulation's starting conditions.

In order to find an optimal solution for the design and implementation of the simulation framework the project is divided into several steps. In a first step detailed requirements to the simulation framework will be elaborated based on the goals described above. In a second step a software design and simulation algorithm will be chosen which fulfill the previously defined requirements. The third step consists of the implementation of the software what will be done in a software development typical iterative manner. The iterations include implementation, test and refinement until all requirements are met. In a final step the framework will be applied to a simplified fermentation process setup and will serve as a proof of concept. The simplified setup contains only Lactobacillus brevis as contamination bacteria. As a measure of the product quality the density of ethanol and lactic acids shall be used. The contamination with Lactobacillus brevis is a typical scenario which can lead to the effect that the beer turns sour under certain conditions [6].

If the project progress allows further development, additional design goals can be implemented like an generic or automated integration of bacteria models, an optimized user interface, a graphical user interface or an enhanced model of the fermentation process.

There have been many approaches to simulate microbial communities [9]. To model the behavior of the simulated bacteria genome-scale models (GEM) are used as their usability in similar applications has been often proven and a variety of models are publicly available [4].

Zomorrodi et al. presents in [9] a comprehensive overview over different simulation approaches of microbial communities. The methods ranges from steady-state models to dynamic models and spatio-temporal models. They all have in common that they use GEMs and flux balance analysis (FBA) to evaluate the behavior of the involved bacteria for certain environmental conditions but they differ in the variety of result aspects and so also in their computational

effort.

Steady-state models like compartmentalized community-level metabolic modeling uses a multispecies stoichiometric metabolic model [5] which is optimized using a mutual objective function. Other approaches as in OptCom use nested optimizations where again a mutual objective function is used to connect the bacteria's models to each other [8]. The principle to use a mutual objective function has the disadvantage that these models can only by applied to microbial communities which have a common objective and do not purely compete with each other [9]. As such a behavior can not be excluded in fermentation processes with bacterial contamination this approach can not be used in this project.

Another requirement is that the simulation algorithm must be able to simulate batch cultures. Steady-state models are only partly usable in such simulations. A better option are here spatio-temporal and dynamic models. Both are able to take the varying densities of metabolites into account. Spatio-temporal models additionally uses spatial distributions of the bacteria to predict their behavior. As such simulations have a very high computational complexity[9] these methods must also be excluded in practical approaches as in this project. Dynamic models use differential equations to embed the bacteria's models into the environment and to predict its temporal dynamics dependent on the bacteria's behavior which is modeled using GEMs and FBA. This approach has already been used in a competitive setup [7] and several implementations with different optimizations of the algorithm are available [4].

Simulating microbial communities is a young and evolving field which enables completely new ways in bio engineering. There have been many applications e.g. in biofuel production [3] [1]. Although in this project a fermentation process shall be the use case this framework is not dedicated to one application. Also other combinations of bacteria GEMs can be simulated with another set of simulation parameters. It builds a basis for future projects and applications and due to its modularity and open-source access it can be enhanced by other scientists and python programmers to adapt it to their own very special applications.

The simulation setup used as proof of concept can be refined and used as a basis to a more enhanced model which can give a more detailed insight into the fermentation process. The results of the simulation leads to new and a more detailed knowledge about the fermentation process and can be correlated to the simulations starting conditions. This knowledge leads to better control of the fermentation process and helps to enhance the quality of the produced beer by reducing the production effort. The gained data can also be used for a better estimation and control of unwanted fermentation byproducts which leads to a longer or refined estimation of the durability of the produced beer. Process enhancements can e.g. lead to a reduction of used chemicals for cleaning.

2 Novelty

Until now GEMs has not been used to investigate the fermentation process of wort, so this project has the capability to provide completely new insights into this process. As beer brewing is applied all around the world and in huge amounts, results in this field can have a big impact on production processes. Additionally, the simulation framework will use only free available and open-source software while the most existing implementations are written in Matlab which is a commercial product and the source code can not be viewed.

As this implementation can be used completely free and is open source it is very easy accessible and can be used in projects with low budges like in smaller companies, start-ups, institutes and academic research and can also be used by students to learn more about this topic. The easy accessibility will facilitate the development in this field and helps to create new solutions.

New insight in the fermentation process of wort will lower the production costs, increase the durability and enhance the quality of the produced beer. Furthermore it has the potential to reduces the need for high aggressive chemicals to clean the production tanks which lowers the

environmental impact.

Interspecies communication and dependencies in microbial co-cultures are widely unexplored and some natural co-cultures are still not culturable due to missing knowledge about their dependencies [2]. Online services provide GEMs to a variety of bacteria. This simulation framework can predict the growth and substrate dynamics of such cultures based on these GEMs. The results can be compared to lab experiments and used to refine the interspecies models iteratively. Also other highly sustainable areas can be addressed with this framework like the development of treatments of human diseases [9], the reduction of uranium concentrations in conterminated areas [7] or to produce biofuel [3] [1].

As most of the existing implementations of simulation algorithms are in Matlab we will have to find new solutions to replace commercial third-party software with open source projects. As the field of simulation of microbial communities is still under research, the available algorithms are error prone and not optimal. As pointed out by Hanson et al. [4] some available algorithms are prone to numerical instabilities. Another important challenge in the implementation of the framework is to find a compromise between a simple and fast implementation and a modular and easily extendable software.

Besides the simulation framework also the simulation setup covers many challenges. To generate realistic data a simulation of a contaminated fermentation needs a lot of knowledge about real substrate conditions. These conditions must be measured in lab experiments or during production and can vary from setup to setup so a stochastic model of the starting conditions must be considered. Also many modelling constants regarding the intake dynamics of metabolites must be measured in lab experiments and can be dependent of the composition of species in the co-culture.

3   Market – Future vision

Simulation results of fermentation processes lead to be deeper knowledge about starting conditions of the fermentation, the fermentation process and the produced beer. This leads to enhanced production processes, lower production costs with increased product quality. Also secondary processes involved in the production line can be refined, e.g. the cleaning of the production tanks, which leads to lower production costs. Enhanced production processes can also be used to enlarge the durability of the produced beer which is especially important for smaller sized breweries and special kinds of beer which can not be drained.

New developments in the field of microbial communities can lead to reduced process costs, e.g. the usage of bacteria to reduce the uranium concentration in contaminated areas [7].

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