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

Describe the project and its objectives

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 yest 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 Brevisas as contamination bacteria. As a measure of the product quality the density of ethanol and lactic acids shall be used. The contamination with Lactobacillus Brevisas is a typical scenario which can lead to the effect that the beer turns sour under certain conditions [3]. 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.

Describe the state of the art in the field

- short overview over available techniques

- discuss advantages and disadvantages of available techniques

There have been many approaches to simulate microbial communities [5]. 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 [1].

Zomorrodi et al. presents in [5] 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 [2] 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 [4]. The principle to use a mutual objective function has the disadvantage that these models can only by applied to microbial communities which has a common objective and does not purely compete with each other [5]. As such a behavior can not be excluded in fermentation processes with bacteria 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 as a very high computational complexity this method must also be excluded in practical approaches as in this project [5]. Dynamic models use differential equations to predict the temporal behavior of the environment where the behavior of the bacteria is modeled using GEMs and FBA.

Describe how the project can advance the state of the art in the field

- The simulation framework:

    - simulation of co-culture is interesting for many areas
    - (list a few examples)
    - this framework is not dedicated to only one setup
    - builds a basis for future simulation projects
    - python has a bit programmers community which makes it easy to customize or enhance the framework
    - framework can be used to implement new approaches

- The simulation results:

    - new and detailed knowledge about fermentation setup
    - better control of fermentation process and product quality
    - new solutions to ensure product quality
    - estimation of product quality (max/min ratio of bad fermentation products)
    - reduction of chemicals for cleaning

2 Novelty

Describe the novelty of the project and how the results from it can be applied to the benefit of society

Novelty:

- GEMs have not been used in the field of beer brewing

- other implementations of algorithms are mostly in matlab and use costly modules

- our approach will be open-source and available for free

Application of results:

- The simulation framework:
    - is completely free available and open source
    - can also be used by students to learn more about this topic
    - can be used by smaller companies (cheaper than matlab)
    - can be interesting by academic research (cheaper than matlab)
    - will enable new development in the field of co-culture simulation
    - will help to create new solutions how to generate metabolites in a co-culture of several bacterias

- The simulation results:
    - The simulation environment and the setup will be available for free
    - can be used by smaller breweries to
        * the quality of their beer
        * reducing costs of production: less cleaning effort, more efficient processes,...
    - reduces the impact on the environment: less usage of chemicals for cleaning

Describe how the project can lead to the development of goods, processes or services

- new production methods due to co-cultures: find here something in the papers

Which technological and design challenges are posed by the project

- Framework:
    - the most implementations are in matlab, ours will be in python
    - python does not provide many libraries as they are available in matlab
    - it will be neccessary to find alternative ways for implementation / adapt existing algorithms to available ressorces in python
    - to gurantee the stability of the algorithm (cite paper)
    - find a compromise between a simple implementation which is still flexible and modular enough that it can be easily extended to refine the simulation setup/environment
    - dynamic simulation using GEMs is still under research
    - there are still many open problems
    - ...maybe you find some disadvantages you could mention here. Maybe some of them could be a implementation goal to solve them...?

- Simulation (setup):
    - a realistic simulation of the conterminated fermentation needs a lot of knowledge about real medium conditions
    - conditions must most probably be measured somehow
    - conditions vary from setup to setup, so a stochastic model of the starting conditions must be created
    - many modelling constants in the dynamic model must be measured as they are not available in publications (e.g. gas saturation, metabolite input ratios / saturation,...)
    - temperatur dependency is often not modeled in GEMs
    - GEMs are not accurate enough to generate realistic data from scratch (results must be compared with real cultures)

3 Market Future vision

Describe financial benefits which the product can create in the market

- Simulation results:
  - The simulation results can be used to enhance production processes to brew beer of higher quality by reducing the costs of production
  - The costs for cleaning can also be reduced

- Framework (co-culture simulation in general):
  - (find stuff in papers)

References

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[3] Frank Vriesekoop et al. "125th Anniversary Review: Bacteria in brewing: The good, the bad and the ugly". In: *Journal of the Institute of Brewing* 118.4 (2012), pp. 335–345. ISSN: 2050-0416. DOI: 10.1002/jib.49. URL: http://dx.doi.org/10.1002/jib.49.

[4] Ali R Zomorrodi and Costas D Maranas. "OptCom: a multi-level optimization framework for the metabolic modeling and analysis of microbial communities". In: *PLoS computational biology* 8.2 (2012), e1002363.

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