Biotech Beer Brewing
Project status report

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1 Project status

To-Do:

- Start with introduction of processes, the figure with the project timeline does not point out the processes well enough
- update table “project processes”
- update figure “project progress”
- update text in this section

1.1 Milestones

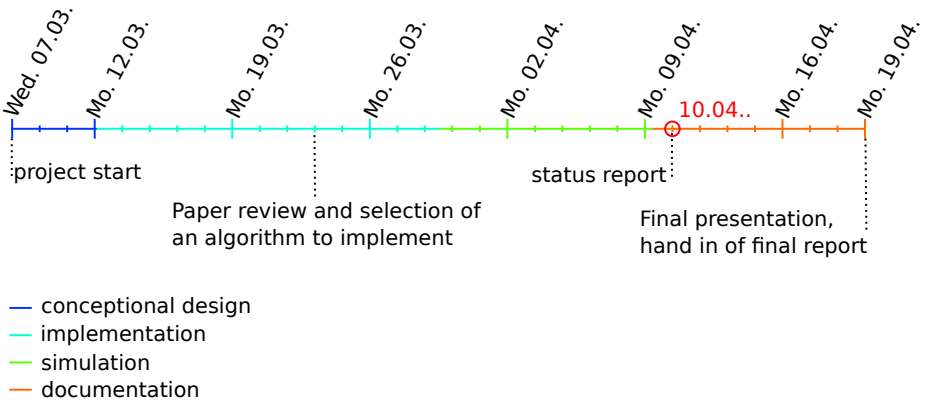

Figure 1: Project timeline

1.2 Progress

The progress of the main project processes in table 1 is illustrated in figure 2. The dashed colored lines show a simple estimated future progress of the processes. If the current trend holds on, the project will be finished approximately 26 working days after the final due date.

Table 1: Project processes

Process	Effort		start date	due date	progress
	Days	Percent			
conceptional design	3.1 d	10 %	07.03.	12.03.	100 %
implementation	12.4 d	40 %	12.03.	28.03.	92 %
research	4.65 d	15 %	12.03.	16.03.	100 %
concept	1.55 d	5 %	16.03.	20.03.	100 %
coding	6.2 d	20 %	20.03.	28.03.	75 %
simulation	7.75 d	25 %	28.03.	09.04.	42 %
research	4.65 d	15 %	28.03.	30.03.	75 %
setup	1.55 d	5 %	30.03.	02.04.	50 %
simulate setup	1.55 d	5 %	02.04.	09.04.	0 %
documentation	7.75 d	25 %	09.04.	17.04.	0 %
analyze results	1.55 d	5 %	09.04.	10.04.	0 %
prepare presentation	3.1 d	10 %	10.04.	16.04.	0 %
prepare report	3.1 d	10 %	16.04.	17.04.	0 %

2 Presentation of intermediate results

2.1 Simulation algorithm

This project will use parts of the Dynamic Multispecies Metabolic Modeling (DMMM) framework to implement dynamic flux balance analysis (DFBA). Table 2 compares selected features of the original implementation of DMMM with the implementation in this project.

Table 2: Overview of implemented features compared to DMMM

Feature	DMMM	This project
Model		
arbitrary many GEMs	yes	yes
arbitrary many metabolites in environment	yes	yes
mortablility of bacteria	yes (in output flux)	yes
input/output flux of bacteria and metabolites	yes	no
parameterized initial state of environment composition	yes	yes
Michaelis-Menten kinetics	yes	yes
Algorithm		
ODE solver	yes	yes
different ODE solvers	yes	no
analytical solver	yes	no

Open Questions:

- How to determin the actual input and output fluxes of the GEMs in one timestep? - What did Zhuang do?

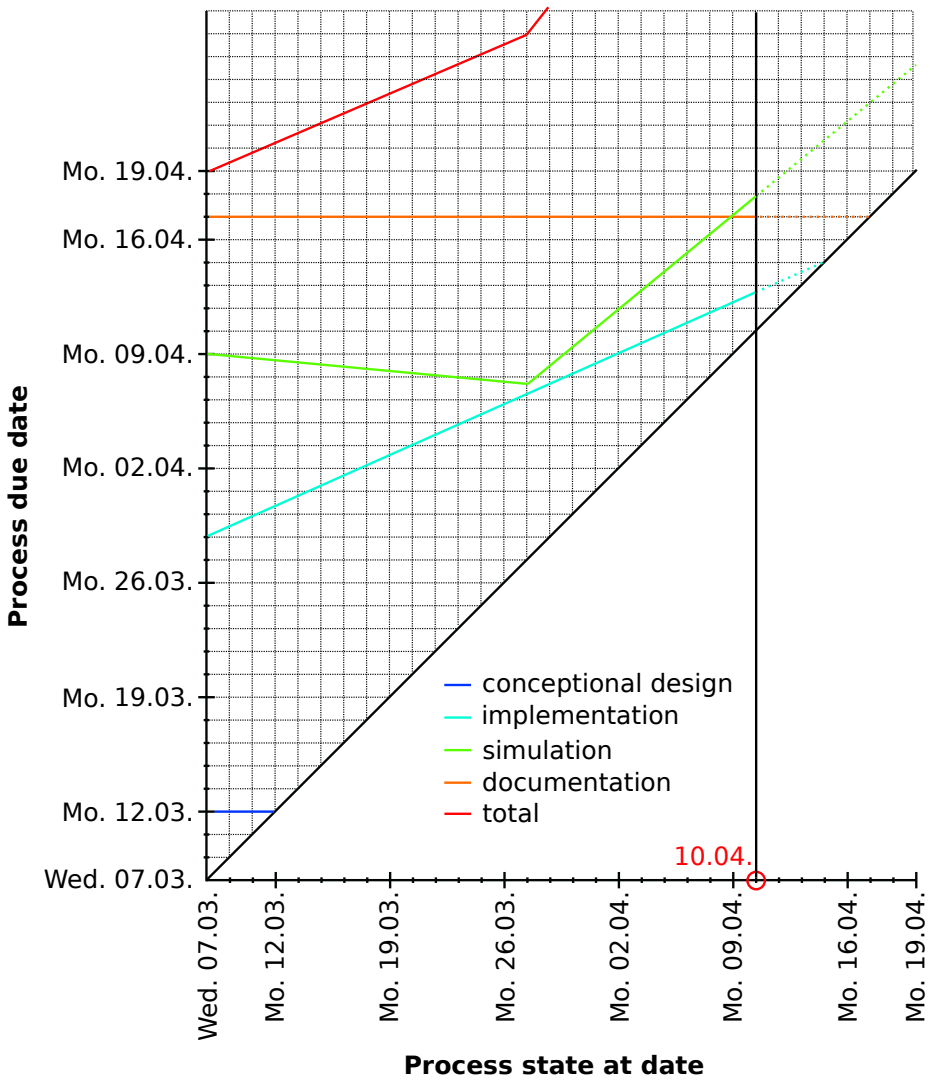

Figure 2: Project progress

As described by Zhuang et al. in [7] the algorithm uses a ODE solver with embedded FBA. A FBA is solved for each GEM in the model and for each time step in the discretised simulation time interval considering the changed metabolite and bacteria densities in the shared environment. The results of the FBAs are used by the ODE solver to solve the differential equations

$$\frac{\mathrm{d}x_j}{\mathrm{d}t} = \mu_j \alpha_j x_j \tag{1}$$

$$\frac{\mathrm{d}s_i}{\mathrm{d}t} = \sum_{j=1}^{N} v_{i,j} x_j \tag{2}$$

which models the dynamics of the bacteria's environment [6] where $i = 1...N$ is the index of metabolites in the shared environment and $j = 1...M$ is the

index of bacteria. The bacteria density is modeled in x_j with $[x_j] = \frac{g}{l}$ and μ_j is the bacteria's growth rate with $[\mu_j] = \frac{mmol}{g_{DW}h}$. $[\alpha_j] = \frac{g}{mmol}$ are constants which defines the weight of one mmol of bactera. Input and output fluxes of the bacteria's models are modeled in $v_{i,j}$ with $[v_{i,j}] = \frac{mmol}{g_{DW}h}$, the densities of metabolites in the shared environment in s_i with $[s_i] = \frac{mmol}{l}$.

In each time step each bacteria's metabolite intake must be changed dependent on the densities of the metabolites in the shared environment. To model saturation of metabolite intake for high metabolite densities Zhuang et al. implemented Michaelis-Menten kinetics [4]

$$v_{max,i,j} = \frac{v_{mm,i,j} s_i}{s_i + k_{mm,i,j}} \tag{3}$$

This formula describes the upper bound of the input flux $v_{max,i,j}$ for metabolite i of bacteria j dependent on the metabolite density s_i. The formula is characterized by to constants $[v_{mm,i,j}] = \frac{mmol}{g_{DW}h}$ and $[k_{mm,i,j}] = \frac{mmol}{l}$ for each bacteria and metabolite.

Mortality is considered using a constant $[\mu_{mort,j}] = \frac{mmol}{g_{DW}h}$ for each bacteria j in this implementation while Zhuang et al. modeled this using the output flux of bacteria out of the system.

Algorithm 1 shows a basic implementation of the differential equations solved by an ODE solver during the simulation similar to DMMM [7].

The algorithm expects a list of bacteria models consisting of

- GEM of this bacteria: A, $\boldsymbol{v_{min}}$, $\boldsymbol{v_{max}}$, $\boldsymbol{w_{growth}}$
- $\boldsymbol{v_{mm}}$ (Michaelis-Menten V_{max}) for each exchange metabolite
- $\boldsymbol{k_{mm}}$ (Michaelis-Menten K) for each exchange metabolite
- α
- mortality μ_{mort}

Furthermore a list of all exchange metabolites in the environment, the bacteria and metabolite densities.

In a first step the upper bounds of the intake fluxes are updated for each bacteria j and exchange metabolite i. The function $update\_intake\_bounds(model_j, s_j, m_i)$ calculates the upper bounds using the formula 3 if the metabolite m_i is contained in $model_j$ as a exchange metabolite and updates this value in the model.

In a next step the GEMs are optimized for growth using FBA, the results are used as growth rate μ_j and actual input and output fluxes $\boldsymbol{v_j}$ of bacteria j in this time step.

The mortality is considered by subtracting the constants $\boldsymbol{\mu}$ from the growth rates $\boldsymbol{\mu}$.

At last step the slopes $\dot{\boldsymbol{x}}$ and $\dot{\boldsymbol{s}}$ are calculated according to 1 and 2 and returned to the ODE solver.

2.2 Simulation Setup

- used GEMs
- why did we choose them? - Is there a reason??

Algorithm 1: Differential equation with embedded FBA

function step($model_1 ... model_M, m_1 ... m_N, x_1 ... M, s_1 ... s_N$);
Input : bacteria models $model_j$, exchange metabolites m_i in environment, bacteria densities x_j, metabolite densities s_i
Output: slope of bacteria and metabolite densities $\dot{x}_j, \dot{s}_i$
for $j := 1$ **to** M **do**
  for $i := 1$ **to** M **do**
    $model_j := update\_intake\_bounds(model_j, s_j, m_i)$
  end
end
for $j := 1$ **to** M **do**
  $\mu_j, \boldsymbol{v_j} := FBA(model_j, \boldsymbol{w_{growth}})$
end
$\boldsymbol{\mu} := \boldsymbol{\mu} - \boldsymbol{\mu_{mort}}$
$\dot{\boldsymbol{x}} := diag(\boldsymbol{\mu})\, \boldsymbol{x}$
for $j := 1$ **to** M **do**
  for $i := 1$ **to** N **do**
    $\dot{\boldsymbol{s}}[m_i] := \dot{\boldsymbol{s}}[m_i] + \boldsymbol{v_j}[m_i] x_j$
  end
end
return $\dot{\boldsymbol{x}}$, $\dot{\boldsymbol{s}}$

- Simulation parameters: V_{max}, K, mortality
- How should the simulation be done?
  - what are the "simulation parameters"?
  - what do we want to measure?
  - What do we want to show?
    - The simulator works
    - Capabilities of this simulations (usage in later applications)

The goal of the simulation is to validate the basic functionality of the simulator using a simplified setup of a realistic future simulation scenario. As defined in our project goals, this simulation scenario is the dynamic flux balance analysis (DFBA) of a co-culture of Saccharomyces cerevisiae and Lactobacillus plantarum.

As genome-scale models a model of Lactobacillus plantarum published by Teusink et al. [5]. A decision about a yeast model is not made yet.

- Input metabolites:
  - Oxygen
    - oxygen saturation of water at 20°C = 9.077 $\frac{mg}{l}$ [2]
    - $s_{init} = 9.077 \frac{mg}{l} = 9.077\ 10^{-3} \frac{g}{l} = \frac{9.077\ 10^{-3} \frac{g}{l}}{18.015\ 10^{-3} \frac{g}{mmol}} = 0,5039 \frac{mmol}{l}$
    - $V_{max,lac}$ =?
    - $k_{m,lac}$ =?

    * $V_{max,sac} = 2.5 \frac{mmol}{g\ h}$ [3]
    * $k_{m,sac} = 0.005mM$ [3]
  - Glucose
    * s_{init}: 5...20 °Plato = $153.031...690.697 \frac{mmol}{l}$
    * [1]
    * $V_{max,lac}$ =?
    * $k_{m,lac}$ =?
    * $V_{max,sac} = 18.5 \frac{mmol}{g\ h}$ [3]
    * $k_{m,sac} = 0.5mM$ [3]

- Output metabolites (considered in results):
  - ethanol
  - ???

3 Outlook

To-Do:

- Update text

Due to unexpected high effort in the research of simulation algorithms and technical problems the project progress is not as fast as expected (see figure 2). It is assumed that the technical problems were a single event and will not occure again, so this will not lead to further delay. As the high effort part in research for simulation algorithms is done, it can also be assumed that this will not cause further delay, too. Under these assumptions (no further delay in all processes) the project delay can be reduced from 12 to 6 days.

To catch up the remaining delay, a higher focus was set to choose a simulation algorithm with less implementation complexity. Furthermore, if this is not enough to catch up the delay, the complexity of the later simulations can be further reduced.

The subsequent steps include:

- research on modeling uptake limits dependent on metabolite densities in the substrate using Michaelis–Menten kinetics
- mathematical formulation of the model
- implementation of the simulation algorithm

References

[1] Zdenek Bubnk et al. *Sugar technologists manual: chemical and physical data for sugar manufacturers and users*. Bartens, 1995.

[2] *Chemical features of water*. 1987. URL: http://www.fao.org/docrep/field/003/AC183E/AC183E04.htm.

[3] Timothy J. Hanly and Michael A. Henson. "Dynamic model-based analysis of furfural and HMF detoxification by pure and mixed batch cultures of *S. cerevisiae* and *S. stipitis*: Dynamic Model-Based Analysis of Furfural and HMF". en. In: *Biotechnology and Bioengineering* 111.2 (Feb. 2014), pp. 272–284. ISSN: 00063592. DOI: 10.1002/bit.25101. URL: http://doi.wiley.com/10.1002/bit.25101 (visited on 03/23/2018).

[4] Kenneth A Johnson and Roger S Goody. "The original Michaelis constant: translation of the 1913 Michaelis–Menten paper". In: *Biochemistry* 50.39 (2011), pp. 8264–8269.

[5] Bas Teusink et al. "Analysis of Growth of *Lactobacillus plantarum* WCFS1 on a Complex Medium Using a Genome-scale Metabolic Model". en. In: *Journal of Biological Chemistry* 281.52 (Dec. 2006), pp. 40041–40048. ISSN: 0021-9258, 1083-351X. DOI: 10.1074/jbc.M606263200. URL: http://www.jbc.org/lookup/doi/10.1074/jbc.M606263200 (visited on 03/27/2018).

[6] K. Zhuang et al. "The design of long-term effective uranium bioremediation strategy using a community metabolic model". en. In: *Biotechnology and Bioengineering* 109.10 (Oct. 2012), pp. 2475–2483. ISSN: 00063592. DOI: 10.1002/bit.24528. URL: http://doi.wiley.com/10.1002/bit.24528 (visited on 03/23/2018).

[7] Kai Zhuang et al. "Genome-scale dynamic modeling of the competition between Rhodoferax and Geobacter in anoxic subsurface environments". en. In: *The ISME Journal* 5.2 (Feb. 2011), pp. 305–316. ISSN: 1751-7362, 1751-7370. DOI: 10.1038/ismej.2010.117. URL: http://www.nature.com/articles/ismej2010117 (visited on 03/23/2018).

4 Appendix

$$d_{total} = 4.13\frac{g}{l\ ^\circ P}p + 997\frac{g}{l} \tag{4}$$

$$d_{total} = \frac{m_{total}}{V_{total}} \tag{5}$$

$$d_{total} = \frac{m_{Glc} + m_W}{V_{total}} \tag{6}$$

$$d_{total} = \frac{m_{Glc} + d_W V_W}{V_{total}} \tag{7}$$

$$d_{total} = \frac{m_{Glc} + d_W \left(V_{total} - V_{Glc}\right)}{V_{total}} \qquad suppose V_{total} = V_{Glc} + V_W \tag{8}$$

$$d_{total} = \frac{m_{Glc} + d_W \left(V_{total} - \frac{m_{Glc}}{d_{Glc}}\right)}{V_{total}} \tag{9}$$

$$s_{Glc} = \frac{m_{glc}}{V_{total}} = \frac{d_{total} - d_W}{1 - \frac{d_W}{d_{Glc}}} \tag{10}$$