Biotech Beer Brewing
Project status report

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1 Project status

1.1 Milestones

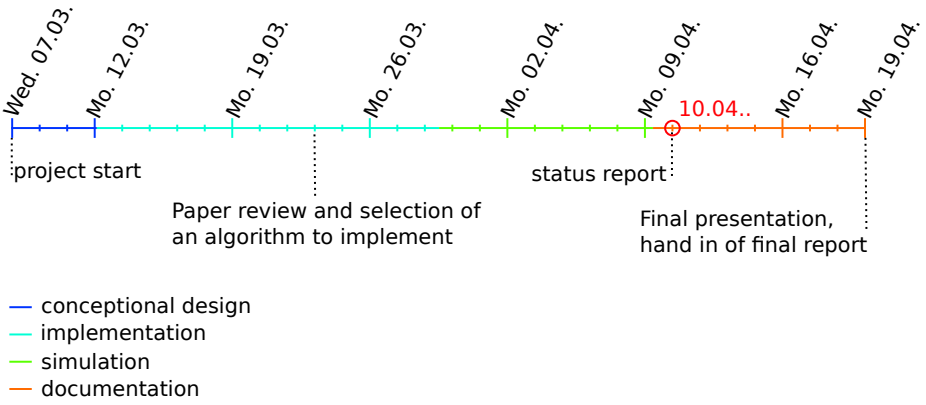

Figure 1: Project timeline

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

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

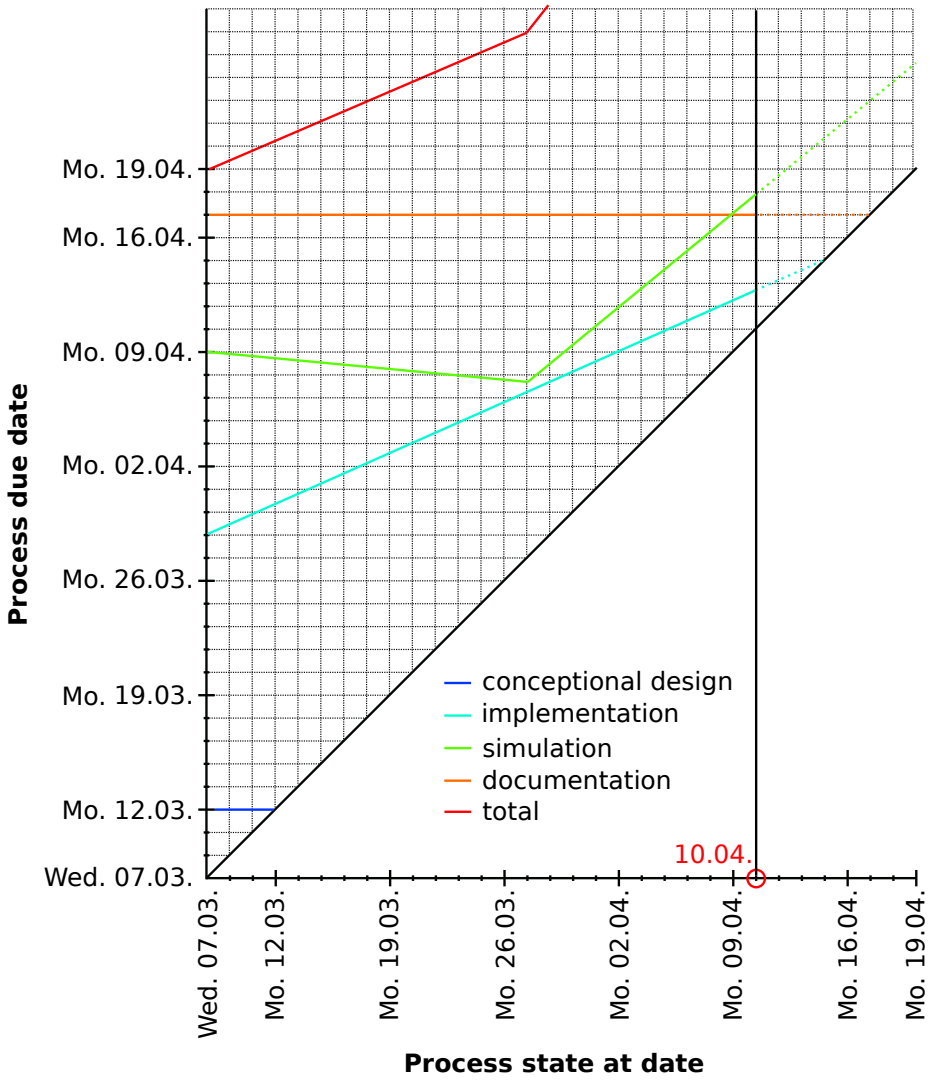

Figure 2: Project progress

FBAs are used by the ODE solver to solve the differential equations

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

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

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 $[\mu_j] = \frac{mmol}{g_{DW}h}$. 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 [3]

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

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 and species
- $\boldsymbol{k_{mm}}$ (Michaelis-Menten K) for each exchange metabolite and species
- 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

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.

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 $\dot{\boldsymbol{x}}$, $\dot{\boldsymbol{s}}$

Table 3: Model constants used in the simulation setup

Constant	S. cerevisiae	L. plantarum
Maximum glucose uptake rate (mmol/g/h)	18.5	?
Maximum oxygen uptake rate (mmol/g/h)	2.5	?
Glucose uptake saturation constant (g/l)	0.5	?
Oxygen uptake saturation constant (mM)	0.005	?

The simulation will consider two input metabolites: oxygen and glucose. Table 3 and table 4 contain all values needed to define the initial metabolite conditions and kinetics.

To verify the basic functionality of the simulator the resulting bacteria densities and metabolite densities of ethanol, d- and l-lactate, oxygen and glucose will be compared to existing data.

3 Outlook

The current delay of 26 days will be compensated with additional shifts and it is assumed that parts of both status reports can be reused in the final report.

The subsequent steps include:

- debugging of simulator code
- research on metabolite kinetics for Lactobacillus plantarum
- choosing a yeast GEM

Table 4: Simulation parameters used in the simulation setup

Parameter	value	reference
Initial glucose density (mmol/l)	272.9 ... 1230.755	equation 6, table 5
Initial oxygen density (mmol/l)	0.5039	equation 7, table 5

- research on reference data to verify simulation results
- start simulations

4 Appendix

The following approximation is used to convert °C ("degree plato") to a density measure (g/l)[1].

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

As the simulation framework expects metabolite densities relative to the total volume of the solution (mmol of metabolite per liter solution, mmol/l) the total density d_{total} must to converted to a density s_{glc}. It is assumed that $V_{total} = V_{glc} + V_W$.

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

$$d_{total} = \frac{m_{glc} + m_w}{V_{total}}$$

$$d_{total} = \frac{m_{glc} + d_w V_w}{V_{total}}$$

$$d_{total} = \frac{m_{glc} + d_w \left(V_{total} - V_{glc}\right)}{V_{total}}$$

$$d_{total} = \frac{m_{glc} + d_w \left(V_{total} - \frac{m_{glc}}{d_{glc}}\right)}{V_{total}}$$

$$s_{glc} = \frac{m_{glc}}{V_{total}} = \frac{d_{total} - d_w}{1 - \frac{d_w}{d_{glc}}} \tag{5}$$

Combining equation 4 and 5, including all constants and converting it to mmol/l leads to:

$$s_{glc} = \left(63.857 \frac{1}{\circ P} p - 46.385\right) \frac{mmol}{l} \tag{6}$$

To calculate the initial oxygen density in the solution it is assumed that the solution is at 20 °C and fully saturated with oxygen:

$$s_{init,ox} = 9.077 \frac{mg}{l} = 9.077\ 10^{-3} \frac{g}{l} = \frac{9.077\ 10^{-3} \frac{g}{l}}{18.015\ 10^{-3} \frac{g}{mmol}} = 0,5039 \frac{mmol}{l} \tag{7}$$

Table 5: Constants used in this document

Constant	symbol	value	reference
Oxygen saturation of water at 20°C (mg/l)	-	9.077	[2]
Molar mass of water (g/mol)	-	18.015	[4]
Molar mass of glucose (g/mol)	-	180.156	[4]
Density of water (g/l)	d_w	1.00	[4]
Density of glucose (g/l)	d_{glc}	1.56	[4]
Typical glucose/water solution density to brew beer (°P)	-	5...20	-

References

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[3] 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.

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[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).