Kinetic models

September 24, 2015

1 Background

In our project we really tried to make the dry lab and the wet lab go hand in hand. To achieve this we tried to solve some problems that we wanted to solve before we would alter strains in the wet lab with the help of genome-scale FBA models. After the (altered) strains were characterized, we used the parameters from physiology measurements for further kinetic modeling of our consortium. These kinetic models help us in making predictions for further applications.

2 Aim

We want to answer questions about the dynamics of an engineered consortium, which will help in envisioning an archetypal final application. What will happen to the growth rate of the organisms during the cultivation? With what initial conditions and parameters will the ratio between the biomass of the different species converge to the same ratio? And if so, what will that ratio be? What is the influence of the light intensity on Synechocystis and how will this be influenced by the presence of a chemoheterotroph which blocks and scatters light? To answer these questions we created kinetic models consisting of ordinary differential equations (ODEs).

3 Approach

We modeled the biomass per liter of *Synechocystis* and Esherichia coli in chemostat as well as batch cultures. We created a set of differential equations and analyzed them with pydstool. A python tool, which can solve differential equations numerically.

3.1 Batch

3.1.1 One-way dependency

Unlimited cell growth is exponentially. The amount of biomass per time for an exponentially growing species can be given by the following different equation:

$$\frac{da}{dt} = \mu a \tag{1}$$

Herein is a the amount of biomass per liter and μ the growth rate normalized for biomass. One can easily verify that the solution of this differential equation is indeed exponential growth ($a = ce^{\mu t}$). Now from experimental data it has been shown that limited growth on a substrate is a bit different. The normalized growth rate is then dependent on the concentration of substrate. According to the monod equation, the μ is dependent on $[S]$ in the following way:

$$\mu = \mu_{max} \frac{[S]}{k_S + [S]} \tag{2}$$

Herein is μ_{max} the maximal growth rate (equal to the growth rate at unlimited growth), and $[S]$ the concentration of substrate. k_S is the concentration of $[S]$ at a rate $\frac{1}{2}\mu_{max}$. We also know this equation from enzyme kinetics as the Michaelis-Menten equation. In enzyme kinetics this equation is used to calculate the rates at which enzymes convert products. However the Michaelis-Menten equation is based on theoretical arguments, while Monod is based on experimental findings. According to Monod, the growth rate saturates as the concentration becomes higher (see figure 1).

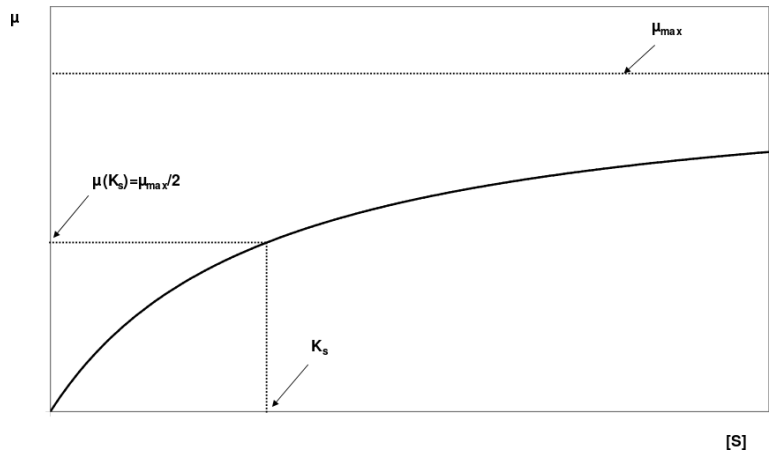

Figure 1: Limited growth on a substrate according to the Monod equation. μ is the normalized growth rate in units per hour μ_{max} is the maximal growth rate and $[S]$ is the substrate concentration. k_S is the concentration at the rate equal to $\frac{1}{2}\mu_{max}$.

In our consortium, Escherichia coli grows limited on acetate. So now we know how μ depends on the concentration of acetate. We now need to model the concentration of acetate. We assume the synthesis of acetate is growth coupled and depends linear on the growth of *Synechocystis*. We also know that the maximal uptake rate of acetate by *E. coli* is dependent $1/y$ herein is y the yield of *E. coli* on acetate in is in milligram Dry Weight *E. coli* per millimole acetate per liter. The uptake of substrate per unit time is also in a a saturable way dependent on the concentration of S, exactly the same way the growth speed is dependent on the the concentration of substrate. The synthesis of the substrate is growth coupled and is dependent on the amount of biomass of *Synechocystis* formed in time. The ch We now arrived at the following set of differential equations:

$$\frac{d\text{syn}}{dt} = \mu_{syn}\text{syn} \tag{3}$$

$$\frac{d\text{ec}}{dt} = \mu_{max,ec}\frac{[S]}{k_{s,ec}+[S]}\text{ec} \tag{4}$$

$$\frac{d[S]}{dt} = \frac{1}{y_{s,syn}}\mu_{syn}\text{syn} - \frac{1}{y_{s,ec}}\frac{[S]}{k_{s,ec}+[S]}\text{ec} \tag{5}$$

Herein is syn the amount of biomass of *Synechocystis* and ec the amount of biomass of E. coli. y_s is the substrate yield of *Synechocystis*. Since in this model the substrate is only formed when *Synechocystis* forms biomass, per amount of biomass formed, there is a constant amount of substrate formed. The yield is usually expressed in gram dry weight mole substrate *used*. In this case we mean gram dry weight mole substrate *formed*. So to find the amount of substrate that is formed per amount of biomass that is formed we simply take $\frac{1}{y,syn}$.

It can be easily seen that such a relationship will not be stable if $\mu_{max,ec} << \mu_{max,cyn}$. In this case by stability we mean the convergence of the growth rate to the same value.

3.1.2 Substrate dependent

In this model, *Synechocystis* is not dependent on E. coli. There are two ways in which *Synechocystis* may be dependent on E. coli we have explored. Firstly, *E. coli* may produce a substrate *Synechocystis* grows on, as is the case with the auxotrophic *Synechocystis*. Secondly, E. coli may decrease the light intensity in the culture, in this way slowing down the growth of *Synechocystis*. The growth rate of *Synechocystis* can only be limited by one of these two processes and it will always be limited by the process that slows it the most. Either μ_{syn} is lower than $\mu_{max,syn}$ because there is a photon shortage, but then the amount of substrate available at that growth rate would be enough, or the amount of substrate is limiting, but then the amount of photons available would also be enough for that given growth rate. So actually the growth rate of *Synechocystis* would be

$$\min(\mu_{max,syn}\frac{[S_2]}{k_{s,syn}+[S_2]}, \mu_{max,syn}f(\text{syn},\text{ec})) \tag{6}$$

Herein is $f(\text{syn},\text{ec})$ a function which determines the factor of decrease in growth rate because of a photon shortage and it is a function of the amount of biomass per liter of *Synechocystis* as well as that of E. coli. If we

now assume that the amount of substrate E. coli produces is going to be limiting we arrive at the following set of differential equations.

$$\frac{d\text{syn}}{dt} = \mu_{max,syn}\frac{[S_2]}{k_{s2,syn}+[S_2]}\text{syn} \tag{7}$$
$$\frac{d\text{ec}}{dt} = \mu_{max,ec}\frac{[S_1]}{k_{s1,ec}+[S_1]}\text{ec} \tag{8}$$
$$\frac{d[S_1]}{dt} = \frac{1}{y_{s,syn}}\mu_{max,syn}\frac{[S_2]}{k_{s2,syn}+[S_2]}\text{syn} - \frac{1}{y_{s1,ec}}\frac{[S]}{k_{s1,ec}+[S]}\text{ec} \tag{9}$$
$$\frac{d[S_2]}{dt} = Q_{p,ec}\text{ec} - \frac{1}{y_{s2,syn}}\frac{[S_2]}{k_{s2,syn}+[S_2]}\text{syn} \tag{10}$$

Herein is $Q_{p,ec}$ the amount of $[S_2]$ formed by E. coli per gram dry weight of E. coli. We assume E. coli doesn't produce in a growth coupled way, but has a constant production per amount of biomass.

$$\frac{d\text{syn}}{dt} = \mu_{max,syn}\frac{[S_2]}{k_{s2,syn}+[S_2]}\text{syn} \tag{11}$$
$$\frac{d\text{ec}}{dt} = \mu_{max,ec}\frac{[S_1]}{k_{s1,ec}+[S_1]}\text{ec} \tag{12}$$
$$\frac{d[S_1]}{dt} = \frac{1}{y_{s,syn}}\frac{d\text{syn}}{dt} - \frac{1}{y_{s1,ec}}\frac{d\text{ec}}{dt} \tag{13}$$
$$\frac{d[S_2]}{dt} = Q_{p,ec}\text{ec} - \frac{1}{y_{s2,syn}}\frac{d\text{syn}}{dt} \tag{14}$$

here

3.1.3 Light limited growth

Like stated in section 3.1.1, it is also possible for the photoautotroph to grow light limited instead of substrate limited. As the cell density of both organism increases, the available light for the photoautotroph decreases. In equation 6 it is already suggested that this light dependency might be a function which is dependent on the biomass of *E. coli* and the biomass of *Synechocystis*. But let's first take a look at light dependent growth. According to literature Franco-Lara et al. [2006], the μ if a photoautotroph is dependent on the light intensity in the following way:

$$\mu(I) = \mu_{max,syn}\frac{I}{K_{s,syn}+I+\frac{I^2}{K_i}} \tag{15}$$

Herein is $\mu(I)$ the specific growth rate on light, $\mu_{max,syn}$ the maximal specific growth rate of *Synechocystis*, I the light intensity, $k_{s,syn}$ is the saturation constant on light and K_i is an inhibitory constant. Now the light intensity is not only dependent on the cell density of the photoautotroph and the chemoheterotroph, but it is also dependent on the place in the reactor. However, to not further complicate the model we made a simplification, by assuming the light intensity is linear dependent on the biomass concentration of *Synechocystis* and *E. coli* in the following way:

$$I(\text{syn},\text{ec}) = \max(I_{max} - sh_s\cdot\text{syn} - sh_e\cdot\text{ec}, 0) \tag{16}$$

Herein are sh_s and sh_e 'shading coefficients' for *Synechocystis* and *E. coli* respectively. In this way the light intensity linear decreases with the amount of biomass of *E. coli* and *Synechocystis*, but it can never be lower than zero. We then arrive at the following set of differential equations:

$$\frac{d\text{syn}}{dt} = \mu_{syn}\text{syn} \tag{17}$$
$$\frac{d\text{ec}}{dt} = \mu_{ec}\text{ec} \tag{18}$$
$$\frac{d[S]}{dt} = \frac{1}{y_{s,syn}}\mu_{syn}\text{syn} - \frac{1}{y_{s1,ec}}\mu_{ec}\text{ec} \tag{19}$$
$$\tag{20}$$

with:

$$\mu_{syn} = \mu_{max,syn} \frac{I}{I + k_{s,syn} + \frac{I^2}{k_i}} \tag{21}$$

$$\mu_{ec} = \mu_{max,ec} \frac{[S]}{[S] + k_{s,ec}} \tag{22}$$

With I the light intensity, dependent on the biomass or *E. coli* an *Synechocystis* as given in equation 16.

3.2 Turbidostat

If we want to model the consortium in a turbidostat, we have to account for the fact that both *Synechocystis* and E. coli are increasing the OD as they grow. This means that the dilution rate is dependent on the biomass of *Synechocystis* as well as that of E. coli. For simplicity we make the assumption that there is a constant flow through the system, instead of only diluting when the threshold is reached. To understand this we first look at the case of a single strain, called b. In a chemostat the growth rate of the organism would become equal to the dilution rate. In a turbidostat however, an organism can grow at its maximal growth rate, but the amount of biomass must still become constant. This means the following:

$$\frac{db}{dt} = \mu \cdot b - D \cdot b = 0 \tag{23}$$

Where b is the amount of biomass of the strain b, mu is the growth rate and D the dilution rate.
In a chemostat it would mean that $D < \mu_{max}$ is a chosen dilution rate and that μ becomes equal to D due to substrate limitation. In a turbidostat however, D becomes equal to μ_{max}, because the species in not growing limited.
In the case where there are two strains, strain a and b, that share a turbidostat, the differential equations that then describe the system looks like the following:

$$\frac{da}{dt} = f(a, b, t) - Da \tag{24}$$

$$\frac{db}{dt} = g(a, b, t) - Db \tag{25}$$

Herein are f and g functions that describe the growth of the organisms a and b respectively. D is again the dilution rate. Now in a shared turbidostat it holds that $a + b = k$, where k is a constant amount of biomass. Then the following holds:

$$a + b = k \tag{26}$$

$$\implies \frac{da}{dt} + \frac{db}{dt} = 0 \tag{27}$$

$$\implies f - Da + g - Db = 0 \tag{28}$$

$$\implies Da + Db = g + f \tag{29}$$

$$\implies D = \frac{g + f}{a + b} \tag{30}$$

For the turbidostat we then arrive at the following set of differential equations:

$$\frac{d\text{syn}}{dt} = \mu_{syn}\text{syn} - D\text{syn} \tag{31}$$

$$\frac{d\text{ec}}{dt} = \mu_{ec}\text{ec} - D\text{ec} \tag{32}$$

$$\frac{d[S_1]}{dt} = \frac{1}{y_{syn/S1}}\mu_{syn}\text{syn} - \frac{1}{y_{ec/S1}}\mu_{ec}\text{ec} - D[S_1] \tag{33}$$

$$\frac{d[S_2]}{dt} = Qp_{S2/ec}\text{ec} - \frac{1}{y_{syn/S2}}\mu_{syn}\text{syn} - D[S_2] \tag{34}$$

where

$$D = \frac{\mu_{syn} + \mu_{ec}}{\text{syn} + \text{ec}} \tag{35}$$

$$\mu_{syn} = \mu_{max,syn} \frac{[S_2]}{k_{s2,syn} + [S_2]} \tag{36}$$

$$\mu_{ec} = \mu_{max,ec} \frac{S_1}{k_{s1,ec} + [S_1]} \tag{37}$$

4 Results

4.1 Batch

4.1.1 One-way dependency

First we simulated the one-way dependent model as depicted by equations 3. To find out whether the system is robust with respect to the initial conditions we did some simulations. Our expectations were that the biomass ratio of the photoautotroph and the chemoheterotroph in this model would converge irrespective to the initial conditions. To find out whether the biomass ratio of this system converges, we did some simulations. To be able to make simulations with the models, we have to estimate and measure the parameters. We tried to measure all parameters as accurate as possible. In table 1 the measured parameters are given.

$\mu_{max,syn}$ (h^{-1})	0.075
$\mu_{max,ec}$ (h^{-1})	0.08
$k_{s,ec}$ (mM)	0.02
$y_{\frac{s}{syn}}$ ($mmol \cdot gDW^{-1}$)	0.40
$y_{\frac{ec}{s}}$ ($gDW \cdot mmol^{-1}$)	0.031

Table 1: Measured parameters for the one-way dependency model. It can be seen that $\mu_{max,ec} < \mu_{max,syn}$. This is highly unlikely the correct value for $\mu max, ec$

It can be seen from this table, that $\mu_{max,ec} < \mu_{max,syn}$. It is very unlikely that this is correct. It might be caused by experimental Since μ of *Synechocystis* higher than the μ_{max} of *E. coli* and given that the growth of *Synechocystis* is independent, we can already expect this model with these parameter to be unstable. *Synechocystis* will keep growing faster than *E. coli*, because the growth rate of *Synechocystis* has a constant value that is higher than the maximal growth rate *E. coli* will ever achieve. If we simulate the model with these parameters find the biomass ratio given in figure 2. It can be seen that the ratio of biomass does not seem to converge. A more clear image can be seen if we look at the *mu*'s of *E. coli* and *Synechocystis* in figure 3.

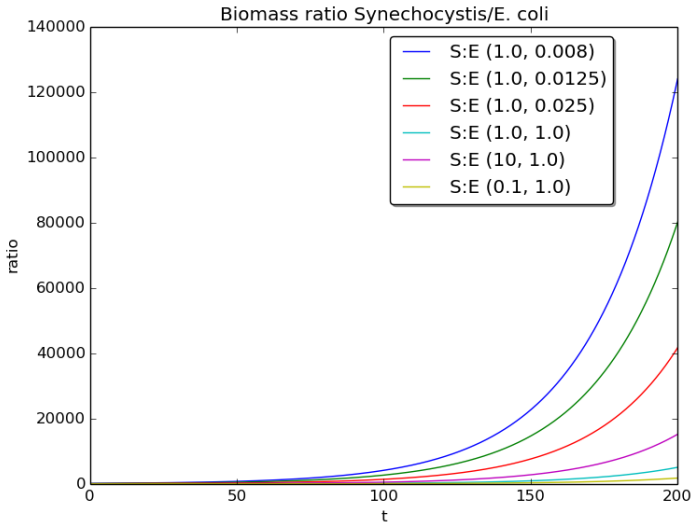

Figure 2: Boiomass ratio's of *Synechocystis*/*E. coli* in time. They seem to diverge, dependent on the initial conditions. This seems logical, since the growth rate of *E. coli* will never become as high as that of *Synechocystis*. In the legend the initial ratio of *Synechocystis* biomass : *E. coli* biomass is given. The model is given by equations 3. Parameters are given by table 1

Chemoheterotrophs are usually have a really high growth rate compared to photoautotrophs. Even though the maximal growth rate of *E. coli* on Acetate might be less than the maximal growth rate on glucose, it is very unlikely that the maximal growth rate is less than that of Synechocystis. So, the question remains, will the biomass ratio converge if we change the parameters such that $\mu_{max,ec} > \mu_{max,syn}$? To test this we have increased $\mu_{max,ec}$ tot 0.08, which is just higher than the growth rate of Synechocystis. In table 2 the new parameters are shown.

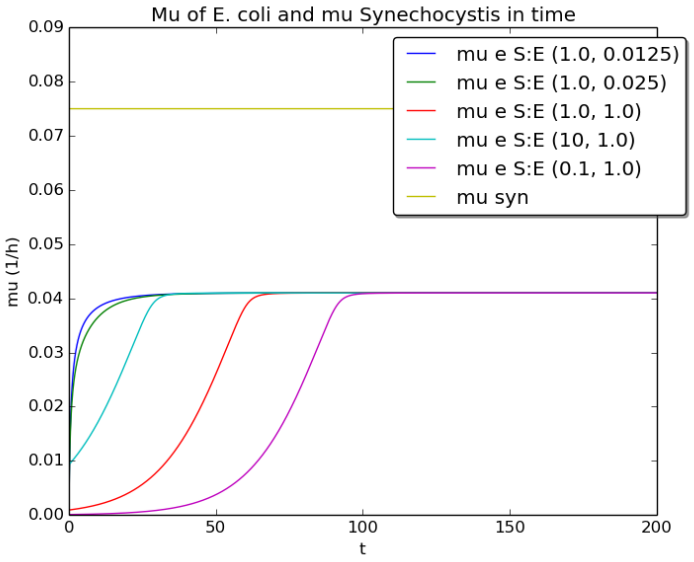

Figure 3: μ_{syn} and μ_{ec} under several different initial conditions (shown in legend in $gDW \cdot l^{-1}$). μ_{ec} converges to $\mu_{max,ec}$, while μ_{syn} stays constant at the value of $\mu_{max,syn}$. The model is given by equations 3. Parameters are given by table 1

$\mu_{max,syn}$ (h^{-1})	0.075
$\mu_{max,ec}$ (h^{-1})	0.08
$k_{s,ec}$ (mM)	0.02
$y_{\frac{s}{syn}}$ ($mmol \cdot gDW^{-1}$)	0.40
$y_{\frac{ec}{s}}$ ($gDW \cdot mmol^{-1}$)	0.031

Table 2: Measured and estimated parameters for the one-way dependency model. It can be seen that now $\mu_{max,ec} > \mu_{max,syn}$.

In figure 4, the biomass ratio over time given several different initial conditions is shown. Now the biomass ratio does seem to converge. However, this ratio is more than ten times higher than what is found in the lab. This might suggest that we still use inaccurately measured parameters.

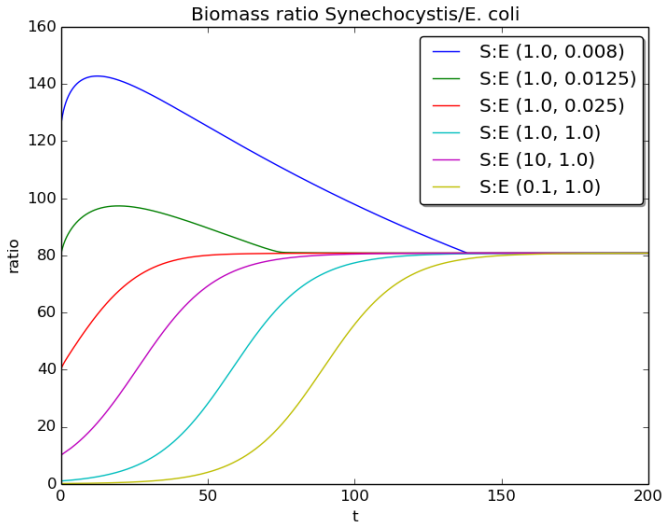

Figure 4: The biomass ratio of *Synechocystis E. coli* under several different initial conditions (shown in legend in $gDW \cdot l^{-1}$). The biomass ratio converges to a constant ratio. The model is given by equations 3. Parameters are given by table 2

If we look once again at the μ's over time in figure 5 we can see that the μ_{ec} converges to μ_{sys}. This is quite intuitive, since the growth rate of *E. coli* is limited by the production of substrate, which is dependent on the growth rate of *Synechocystis*. And the biomasses can only converge to a stable ratio, if the growth rates of both species converge to the same value. We can conclude that the system now is robust with respect to the initial values.

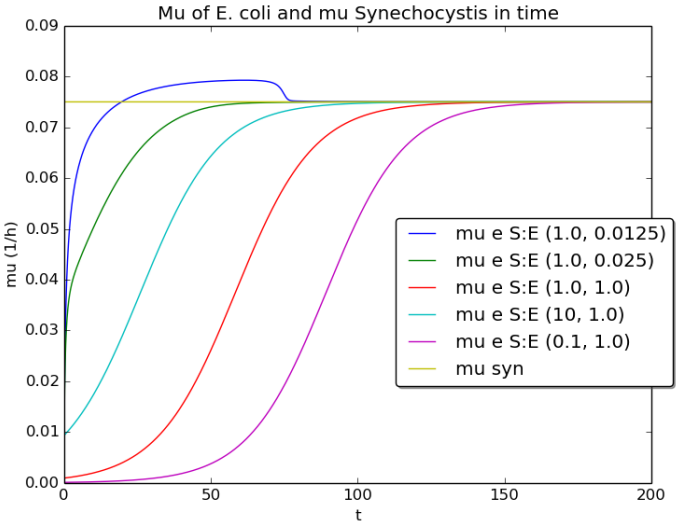

Figure 5: μ_{syn} and μ_{ec} under several different initial conditions (shown in legend in $gDW \cdot l^{-1}$). The μ_{ec} converges to $\mu_{syn} = \mu_{max,syn}$. The model is given by equations 3. Parameters are given by table 2.

4.1.2 Substrate interdependency

To simulate the interdependency on substrates, we needed a lot more parameters. For some of the parameters we had no good measurements, so we estimated them. The general behavior stays generally the same under

$\mu_{max,syn}$ (h^{-1})	0.075
$\mu_{max,ec}$ (h^{-1})	0.08
$k_{s1,ec}$ (mM)	0.02
$k_{s2,syn}$ (mM)	0.02
$y_{\frac{s1}{syn}}$ $(\mathrm{mmol} \cdot \mathrm{gDW}^{-1})$	0.40
$y_{\frac{ec}{s}}$ $(\mathrm{gDW} \cdot \mathrm{mmol}^{-1})$	0.031
$y_{\frac{s2}{syn}}$ $(\mathrm{mmol} \cdot \mathrm{gDW}^{-1})$	0.1
$Q_{p,ec}mmol$ $(\mathrm{mmol} \cdot \mathrm{gDW}^{-1} \cdot \mathrm{h}^{-1})$	0.13

Table 3: Measured and estimated parameters for the substrate interdependent model.

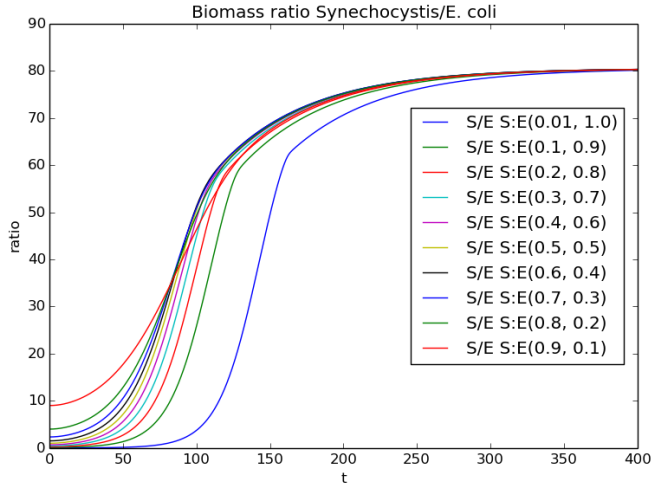

Figure 6: The biomass ratio of *Synechocystis E. coli* under several different initial conditions (shown in legend in $gDW \cdot \mathrm{l}^{-1}$). The biomass ratio converges to a constant ratio. The model is given by equations 7. Parameters are given by table 3

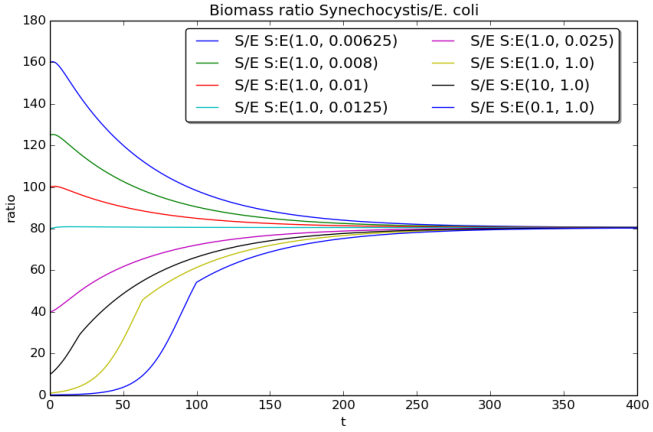

Figure 7: The biomass ratio of *Synechocystis E. coli* under several different initial conditions (shown in legend in $gDW \cdot \mathrm{l}^{-1}$). The biomass ratio converges to a constant ratio. The model is given by equations 7. Parameters are given by table 3

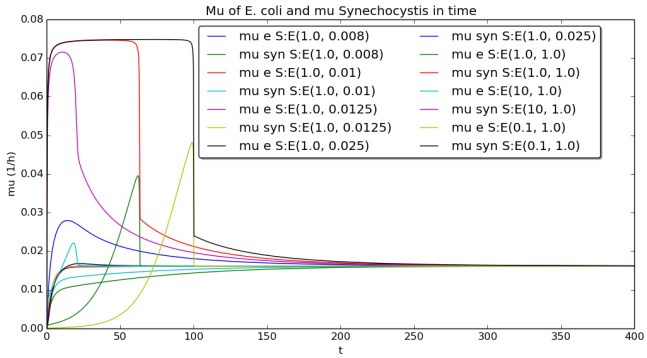

Figure 8: μ_{syn} and μ_{ec} under several different initial conditions (shown in legend in $gDW \cdot l^{-1}$). The μ_{ec} and μ_{syn} converge both to the same value. The model is given by equations 7. Parameters are given by table 3.

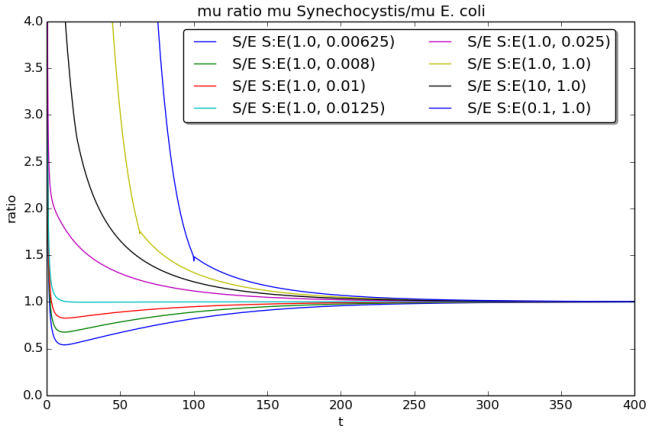

Figure 9: Ratio between μ_{syn} and μ_{ec} under several different initial conditions (shown in legend in $gDW \cdot l^{-1}$). The μ_{ec} and μ_{syn} both converge to the same value, so the ratio converges to one. The model is given by equations 7. Parameters are given by table 3.

4.2 Turbidostat

In a turbidostat you can assume the growth won't be light limited, since every time a threshold OD is measured, the culture is diluted. This means you can regulate the light intensity in such a way, it won't become limiting. This is why we chose to mainly look at the consequences of growing a consortium with a substrate dependent photoautotroph in a turbidostat. So the model we use is described in equations 31 to 37 in section 3.2. The parameters used are given in table 3 In a turbidostat it is expected that the total amount of biomass, so that if *E. coli* and *Synechocystis* combined would stay constant. To test this, we have plotted the total amount of biomass under different initial conditions. The result is show in figure 11, and we can see that this is indeed constant.
This however, does not mean that the amount of biomass of each species stays the same. In figure 10 several examples of the amount of biomass in time of *Synechocystis* and *E. coli* are given.

Now we can once again look at the biomass ratio to see if this converges. In figure 12 the biomass ratio in time is given under different initial conditions.

We can see that indeed the biomass ratio converges. Also if we look at the values for μ in figure 14 in time and the ratio of *mu* in time in figure 13, we can see that the growth rates converge. In a turbidostat the dilution rate will become equal to this final growth rate. We can conclude that this system is also robust with respect to the initial conditions.

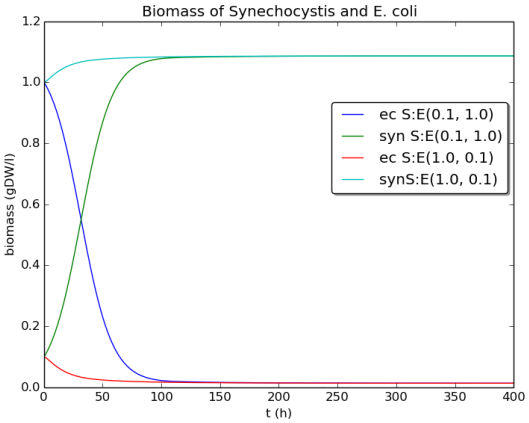

Figure 10: Biomass of *Synechocystis* and *E. coli* in time in a turbidostat. Even thought the total amount stays the same, the amount of each species varies in time.

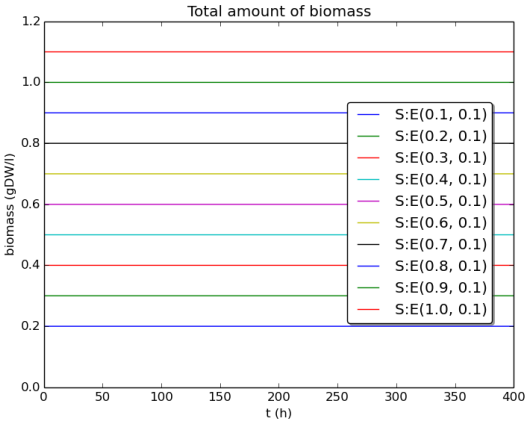

Figure 11: The added biomass of *Synechocystis* and *E. coli* in a turbidostat. Like expected this amount stays constant.

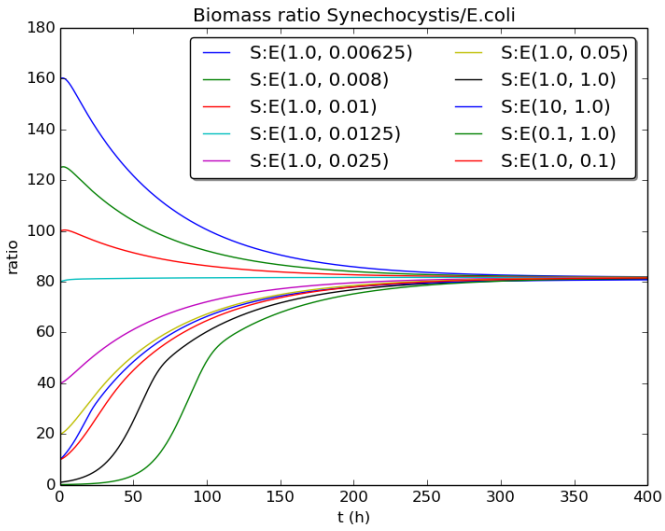

Figure 12: Ratio of the biomass of *Synechocystis* and *E. coli* under several different initial conditions (shown in legend in $gDW \cdot l^{-1}$) in a turbidostat. The biomass ratio of *Synechocystis* and *E. coli* converge to the same value for each initial condition. The model is given by equations 7. Parameters are given by table 3.

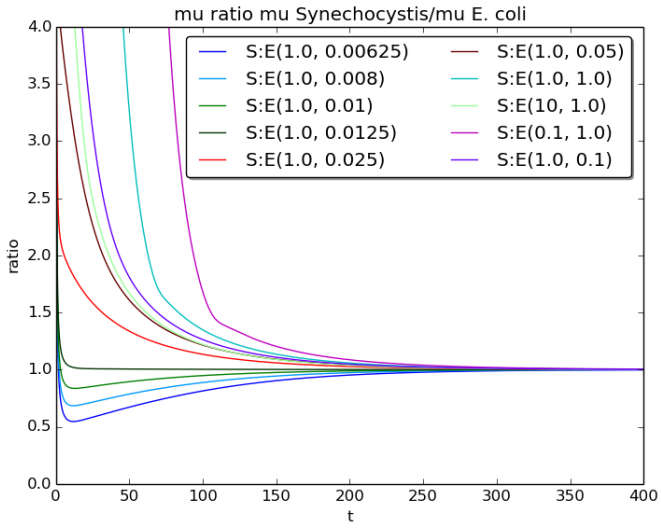

Figure 13: Ratio of μ_{syn} and μ_{ec} under several different initial conditions (shown in legend in $gDW \cdot l^{-1}$) in a turbidostat. μ_{ec} and μ_{syn} both converge to the same value, so the ratio converges to one. The model is given by equations 7. Parameters are given by table 3.

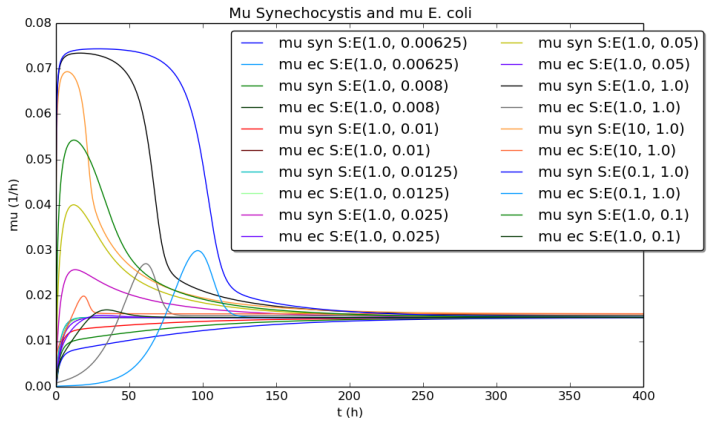

Figure 14: μ_{ec} and μ_{syn} in time in a turbidostat. μ_{ec} and μ_{syn} both converge to the same value, this value is equal to the dilution rate D. The model is given by equations 7. Parameters are given by table 3.

References

Ezequiel Franco-Lara, Jan Havel, Frank Peterat, and Dirk Weuster-Botz. Model-supported optimization of phototrophic growth in a stirred-tank photobioreactor. *Biotechnology and bioengineering*, 95(6):1177–1187, 2006.