Mathematical modelling of stable consortia of light dependent Synechocystis and E. coli. (Module setup)

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Abstract

1 Research question

What type of consortium between Synechocystis and E.coli can be stable and under what constrains?
Subquestions:

- What type of consortium with accompanying genetically engineered pathways can be stable?
- Under what initial conditions and constrains?
- Can the consortium be stable while also producing a product in a sustainable manner?
- (How can the product formation of E. coli be optimized using a consortium of Synechocystis and E. coli?)
- (Which ones are stable under evolutionary pressure?)

1.1 Stability

The main research question focuses on stability. But what exactly do we mean with stability? We can look at stability from three different perspectives. We can look at a mathematical perspective, a population dynamics perspective and from the perspective of the different possible consortia we actually want to make.

When a model consists of a set of differential equations we can do a stability analysis of the equilibria and steady states of the system. This can help us in the analysis of our synthetic consortium, because there may be cases in which the dynamics of the populations of our species are in steady states, or if we have steady state growth and we want to analyze the production of substances with for example a flux balance analysis. However this is a narrow definition of stability, and there are more forms of stability we want to analyze.

In population dynamics, interactions between species can also be modelled with a set of differential equations, but there we are not only interested in the steady states. In predator-prey models for example, periodic solutions can also be really interesting. Periodic solutions will never reach steady state, but you could argue that these periodic solutions are also stable when the system convergence to these solution. Because the species keep on co-existing and after a small perturbation the system will go back to the periodic solution. So then stability seems to have to do something with sustained co-existing of multiple species and convergence of a system to certain solutions.

We might however argue that this definition is too broad. We do not only want two species to co-exist, because then a model of two species competing for the same substrate might then also be interesting and stable. But we are interested in synthetic consortia, which means there must be an interdependence of the two species. This interdependence can be obtained in several ways and so it can also be included in the model in several ways, but it has to be included in some way. Also, because we want to make a sustainable synthetic consortium, one of the species will be the light dependent Synechocystis. Because we want to make it sustainable we want to use this light as an energy source and we want an uptake of CO_2 by the system. A system may be stable with Synechocystis producing carbon products and E. coli producing CO_2 which can then be used by Synechocystis again, but then there is no nett uptake of carbon, which we preferably would want (see section 3.1). So we preferably want the system to have a nett flux from CO_2 and light to some product, preferably produced by E. coli. The model has to be a tool with which these property demands can be researched.

Then there is a fourth form of stability which we haven't considered so far: genetic stability. Over time a loss-of-function, or a gain-of-function mutation might cause one of the species to become independent of the other species, which might not always be a problem, but sometimes it can cause the consortium to fail. For example one of the species might disappear, or the product formation may be stopped. We can analyze the chance of these genetic changes and the consequences. In first instance we will not take this form of stability into account. We will first assume that the species will have a constant genome. But if we want to make a system which is usable for long times and which might have applications in industry we have to consider this form of stability, and engineer our system accordingly.

2 Why Consortia? (under construction)

- More robust in dynamic environments, because there are more available pathways.
- Mixed populations can perform more complex tasks for which compartmentalization is needed. Each member of the consortium can be a specialist in a different task. Balancing two tasks which might complicate each other may be difficult for one species. Also a process that requires different steps can be done easier by consortia.
- Brenner et al. [2008]
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3 Why Synechocystis and E. coli? (Under construction)

Creating substrate out of light CO_2 and H_2O is more sustainable than adding substrate to the medium. We want to be able to use Synechocystis as a green engine to make other organisms able to grow on photosynthetic compounds Synechocystis produces. This can only be one part of the symbiosis, otherwise it won't be stable. It would be a huge evolutionary disadvantage for Synechocystis to 'give away' products while getting nothing back. So in another way Synechocystis must be dependent on E. coli, or E. coli must predate Synechocystis, or some other interaction is needed for this to be stable. E. coli has been the favorite lab pet for the last decades. This means that a lot of tools and applications have been made utilizing E. coli. If we look at iGEM projects alone, a lot of biobricks have been created for E. coli. With our consortium we will be able to utilize all these biobricks created for E. coli in a more sustainable way at once!

3.1 Sustainability (under construction)

In these times humanity faces several great challenges. We might think that we will always be able to live like we were used to on our planet, but considering our own population dynamics (see figure 1) and the accompanying demand for energy and food. We can safely assume that using resources we cannot replenish, will not be a stable strategy and sooner or later one of the irreplaceable resources will run out. When that happens we have to see whether the other resources are able to fill in the gap. Until that happens we have to look for more sustainable ways of producing food and energy. Resources that cannot run out fast, such as sunlight or geothermal energy, have the advantage that they will be a reliable energy source for as long as humanity lives on this planet.

In the way we are using the recourses of the planet now we also have multiple environmental issues. Pollution of the environment may give an advantage on the short term, but polluting strategies are certainly not feasible in the long run.

Then there is also the problem of global warming. Because of the use of fossil fuels a lot more CO_2 is released in the atmosphere and CO_2 levels have been rising. Although CO_2 is not the fastest warming greenhouse gas, a slight change in the balance of gases in the atmosphere, can have an effect on temperature. This slight effect can also have an effect on other sources of greenhouse gases, such as release of methane from permafrost tundras which are now defrosting, enhancing the greenhouse effect. This temperature shift, and gas-compositions shift will have major ecological and economical consequences on our planet. If we want to slow this process down it is of major importance that we reduce the emission of greenhouse gases and CO_2.

This is were the biobased economy comes in. More and more entrepreneurs and scientists are looking for less polluting ways to produce, where possible also with less CO_2 emission. They can do this with the help of biotechnological advances. In biotechnology, there are also still a lot of challenges. In the past food crops have been used to create fuel in a biobased manner. This caused the crop prices to rise in certain areas, which is

seen as a great disadvantage. We have to find a way to produce in a sustainable manner, without competing with food. By choosing a non crop organism, which harvests light and CO_2, like Synechocystis, we might get a small step closer to reach this goal.

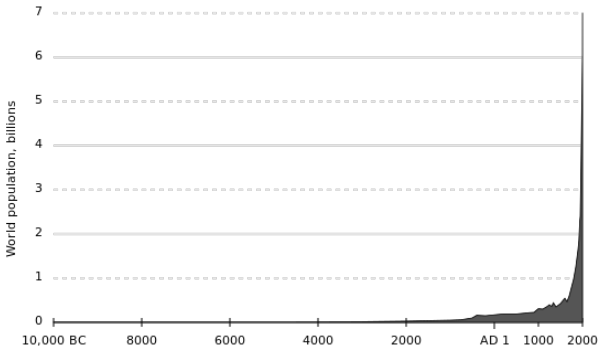

Figure 1: Our own population dynamics so far.

4 Research plan (Under construction)

We can create FBA models for both Synechocystis Fu [2009] and E. coli and simulate the pathways which are necessary for the interactions. Then we can use these parameters in an overall model which can consist for example out of Monod equations. In this monod equations the specific growth rate of Synechocystis must be dependent on light. The amount of light available will be dependent on the population size of E. coli and Synechocystis together. Instead of Monod, can also be a novel method as suggested for example by Wang et al. [2013]. This method uses a kind of costs- and benefits analysis and is especially created for synthetic symbiosis. Kambam et al. [2008] seem to suggest bifurcation analysis and Yukalov et al. [2012] suggests to look at carrying capacity. Parameters like yields and production and consumption rates will be measured in the lab. It is key to find out as soon as possible what parameters we will need to measure The ultimate goal is also to predict what kind of consortium can be stable and check with the lab if this was indeed the case.

4.1 Current plan

We have chosen to do a community FBA. These FBA models can even do dynamic simulations, so the models do not have to assume steady state.
We will first assume that the product formed by E. coli is ethanol and the carbon course Synechocystis produces to be acetate. Questions for the experiments:

- Individual uptake/excretion rates of carbon courses, ethanol, CO_2 and nitrogen and growth rates, QP's, yields of each species?
- How to determine these parameters in a co-culture?
- Which metabolite/s is/are produced by Synechosystis that E.coli uses as substrate?
- Are there other substrates in the medium (possibly produced or consumed by Synechocystis) that E. coli uses as a substrate?
- What is the media composition?
- What other products is E. coli supposed to produce, besides ethanol?

4.2 Steps to take

We will do a community FBA. There has just been created a toolkit with which a dynamic community FBA model can be created by Brett.
To start the following things need to be achieved (deadline may 12):

- Installation of cmbpy toolkit
- Normal FBA analysis of existing model to get familiar
- Collect existing data (xml files) of Synechocystis and E. coli
- Collect existing data for community FBA
- Reading: http://www.nature.com/nbt/journal/v28/n3/full/nbt.1614.html - general FBA; http://www.ncbi.nlm.nih.gov
- Try to reproduce published results, play around with rates and how they affect the solution (e.g. How does the growth rate change if you vary the uptake rates?, What happens to E.coli's growth rate if you force a certain CO2 export?, Can E.coli grow if the oxygen uptake rate is 0?), use different objective functions. etc.
- Meeting with Timo, who can help.

Further steps:

- Having community FBA model running without errors
- Adapting the model to Synechosystis and E.coli (acetate cross-feeding)
- Publish preliminary simulations.
- Collecting experimental results.
- Adapting the model to experimental results.
- Adapting the model to other C-courses based cross-feeding.
- Adepting the model to other mechanisms than cross-feeding.
- Simulations and comparing stability
- Simulations and production optimization
- Pulblish all the things!

4.3 Experimental setup depenence

The research plan also depends on the experimental setup. In FBA there are usually steady state assumptions. These assumptions do not hold when Synechocystis and E. coli are grown in batch cultures. Secondly there was a short mention that our setup might include a spatial separation of E. coli and Synechocystis. Literature seems to suggest these spatially separated consortia might be more stable Kim et al. [2008]. In that case we even might need a spatial model accommodated with the associated partial differential equations. This makes the model more complicated to analyze. Harcombe et al. [2014] seem to suggest that allocation of symbionts is key, but that FBA can still be useful.

5 Place of this module in over all project

As just explained the type of experimental setup will have an influence on the type of model we use. But once the type of model and the general experimental setup is decided, it is key we know as fast as possible what parameters we need to measure, because other modules will take care of these measurements. Once these parameters are known we can play with the model and predict under what constrains a stable consortium can be formed. These constrains will than influence the experimental setup of other modules again. So the modeling and the experiments are in a interdependent loop. We have to be careful here because we don't want to have to wait for other modules. We still have to be able to continue modeling even though we do not yet know all parameters (we can make assumptions and estimates and adjust them later) and on the other hands experiments have to be able to continue without results from the modelling (part of their research questions have to be independent of modelling predictions).

6 Questions for other modules

What is the general experimental setup? (Batch/chemostat, separation of cultures, Synechocystis on the outside etc.)

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