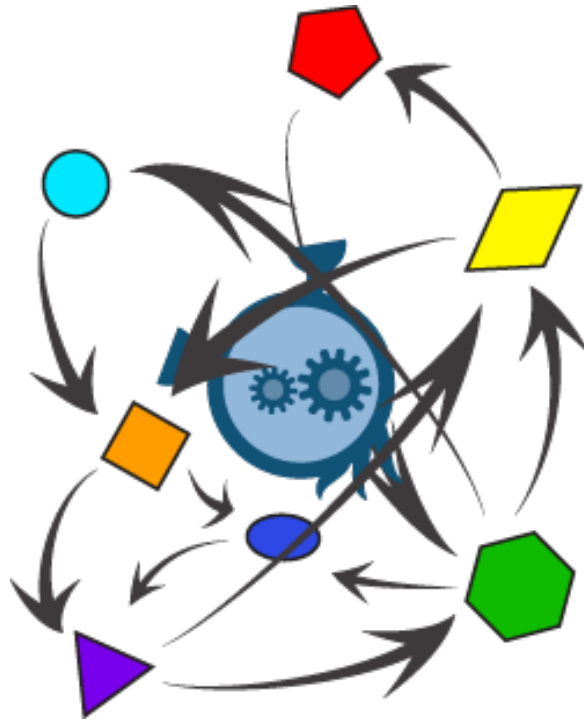


OSCAR OPTIMIZER 1.0

MANUAL



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About

In order to better implement OSCAR as a bioreactor system, it is important that we have a mechanism by which we can optimize his newly developed metabolic network. To achieve this we turned to flux variability analysis as a way of predicting ways of up-regulating our target pathways (such as hydrocarbon production). We developed a MATLAB based program for predicting metabolites that can added to your media in order to increase production of compounds in synthetic *E. coli* chassis'. In addition we have made this program user friendly by designing a graphical user interface, and allowing for other teams to add their own synthetic pathways into the model. We validated this model in the wetlab to demonstrate that it can be used to optimize the Petrobrick system to save time, money, and resources.

What Are We Trying To Model?

Because flux balance provides an easy method to look at how metabolic pathways can be modulated by their inputs. What chemicals can be added into a solution in order to upregulate a synthetic pathway we are introducing into *E. coli*? If we could develop a tool to make this kind of modelling possible it would benefit numerous iGEM teams. To do this, we need to specifically model the flux rate of metabolic pathways responding to different growth media conditions and generate an optimal set of metabolites that should be added to growth media in order to improve production rate.

How Could Systems Like OSCAR Benefit From the Model?

Same as chemical reactions need optimal environmental conditions to achieve maximum production rate, microbes also require optimized growth conditions to accomplish their tasks in maximum speed. During industrial scale up, the optimal conditions for production needs to be maximized while reducing cost of production to a minimum. In microbiological bioreactor systems the conditions of growth media is much more crucial than in chemical synthesis reactors. Furthermore, the selection of media compounds is one of the most significant conditions for growth media and selecting a mix of compounds is very important for this process. If a model can predict an optimal set of metabolites that need to be added into media, this will save time, resources, and funds.

How Does The Program Work?

This program is built upon constraint-based reconstruction analysis and flux variability analysis. It uses the published *E. coli* iAF1260 and *E. coli* core models provided from the Palsson Group University of San Diego. Using this as a base, we constructed reactions and metabolites for our hydrocarbon production component of our project. Specifically, new reactions corresponding to the Petrobrick as well as the upgrading (desulfurization and denitrogenation) pathways were engineered into the *E. coli* base chassis. By running flux variability analysis, program will give different sets of flux rates based on distinct constraints. Finally, the program will analysis the data with an algorithm to generate a set of media compounds that is expected to accelerate production rate.

Setup

SBML Toolbox setup

1. Download SBML Toolbox from [HERE](#).
2. Unzip the toolbox file
3. Install:
 - a. Start MATLAB/Octave
 - b. Navigate to the SBMLToolbox/toolbox directory
 - c. Run the install.m script found in that directory
 - i. Type install in MatLab console

Cobra Toolbox setup

1. Download SBML Toolbox from [HERE](#).
2. Unzip the toolbox file
3. Install:
 - a. Start MATLAB
 - b. Navigate to the Cobra folder Path
 - c. Run initCobraToolbox
 - i. Type initCobraToolbox in MatLab console
 - d. Add the COBRA Toolbox directories to your MATLAB path
 - e. Set the solver
 - i. Type changeCobraSolver in MatLab console
 - f. Type testAll in MatLab console to verify installation

Basic

Pathway build tool

The pathway build tool is an interface to help users to reconstruct the original SBML model. Users can create interested pathways and add them into the model via this interface easily.

Pathway analysis tool

The pathway analysis tool is an interface to help users to analysis pathways and get suggested compounds for growth media to improve production. Users are able to select interested pathway for test, to enter parameters, and to view and export results.

Algorithm

Conceptualization

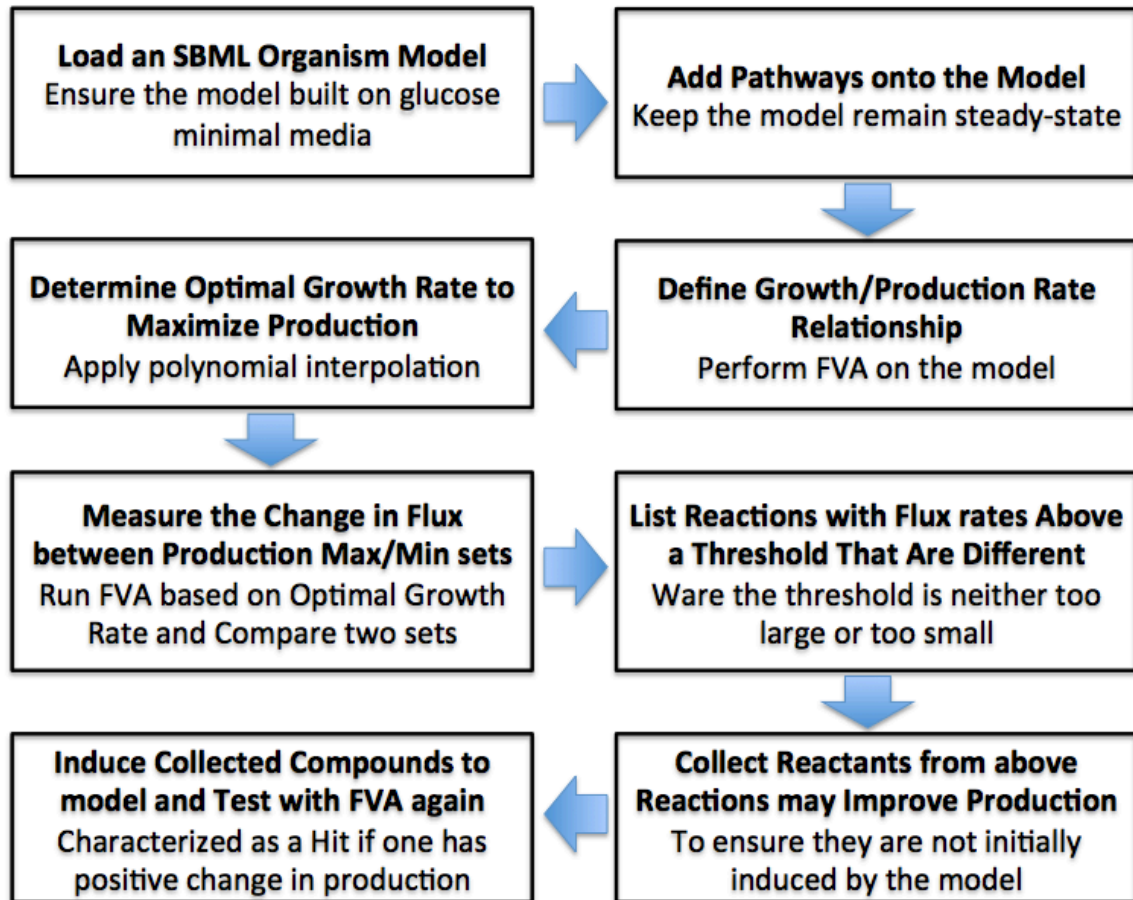
FVA can determine the full range of numerical values for each reaction flux within the network. Additionally, it allows for a better quantification of growth and production rates. Since biomass rate reflects the growth condition, cells must have positive values of biomass flux rate in order to survive and proliferate. This positive growth rate is indicative of a real system as cells are optimized to prefer increases in growth than increases in product output. On the other hand, our goal is to increase the production flux rate above a zero value. This implied among all possible set of fluxes, the optimal flux set should locate a place where growth rate multiplies production rate is maximum.

The algorithm is designed to determine the optimal flux rate of biomass and the value would be set as a new constraint of biomass. Then flux variability analysis would identify the full range of numerical values for each reaction flux within the network that was restricted to the new biological objective (i.e. identify the pathways that are effected to optimize the synthetic pathway of interest).

The differences of values for each reaction in a set of fluxes that maximize and minimize production rates became interesting. By comparing these, some reactions had higher flux rates in production maximum set than production minimum set, some were higher in production minimum set than production maximum set and some had opposite flux directions as most of biological reactions were reversible. These mapped to reactants that would directly effect these reactions based on their quantities. Consequently, the question became how to identify metabolites that by increasing their quantities would improve the production rate.

One of the possible solutions could be comparing two sets of fluxes, determining differences of each reaction between two sets and changing constraints according to reaction needs. For example, if a metabolite needs more in production maximum set than production minimum set, then add more amount of this metabolite by change constraints to improve the production. However not all substrates can be uptaken by the cell or therefore absorbed from the growth media. Hence, only the metabolites that had natural transporters in cell were considered in the final output. Last but not least, to improve production by adding more metabolites to growth media, the analysis should start from a model that was built upon glucose minimum growth media.

Model Step



Application

Building Tab

Analysis Tab

Credits

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