DRY LAB

Team iGEM | IIT Bombay

FLUX BALANCE ANALYSIS

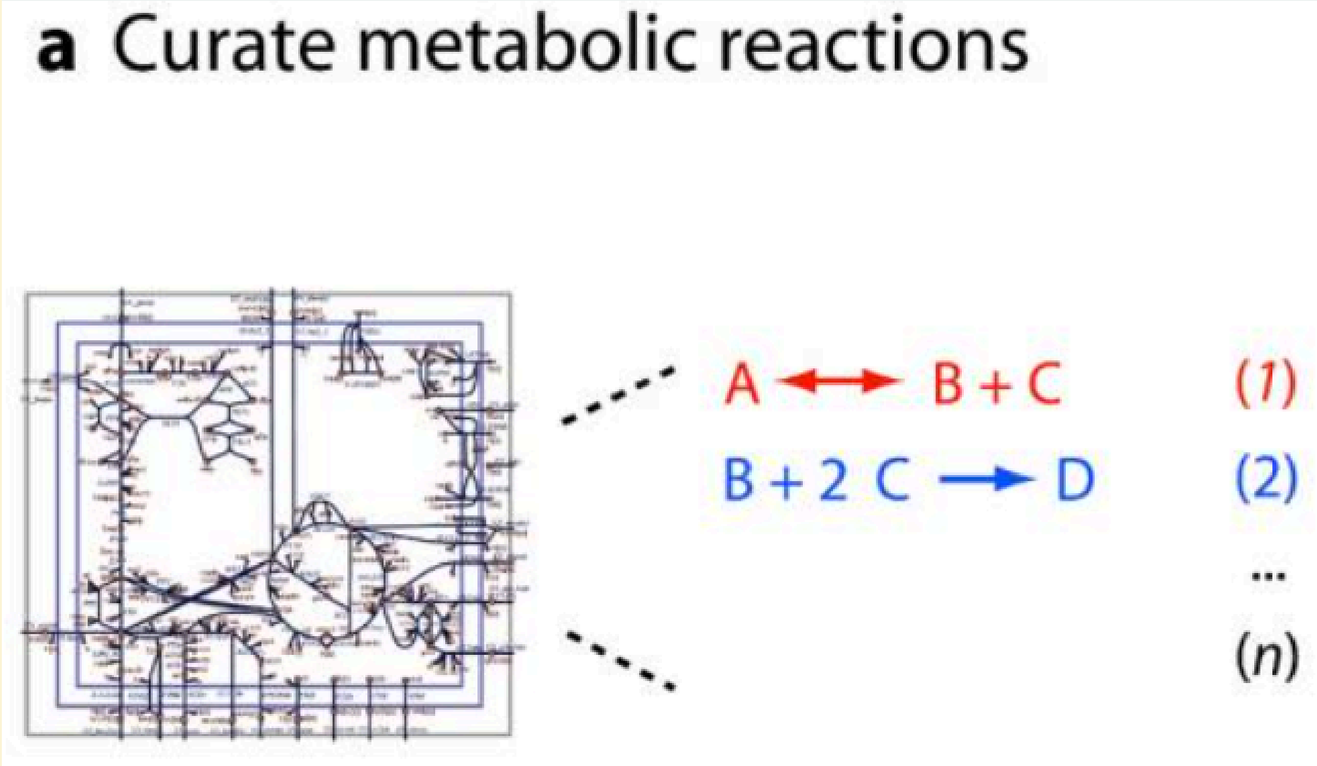
Flux balance analysis is a mathematical approach for analyzing the flow of metabolites through a metabolic network. This makes it possible to predict the growth rate of an organism or the rate of production of a biotechnologically important metabolite. It doesn't involve kinetics and thus, eliminates the need of knowing the rate expressions for various reactions being studied.

link to paper

BASIC STEPS FOLLOWED

Step I

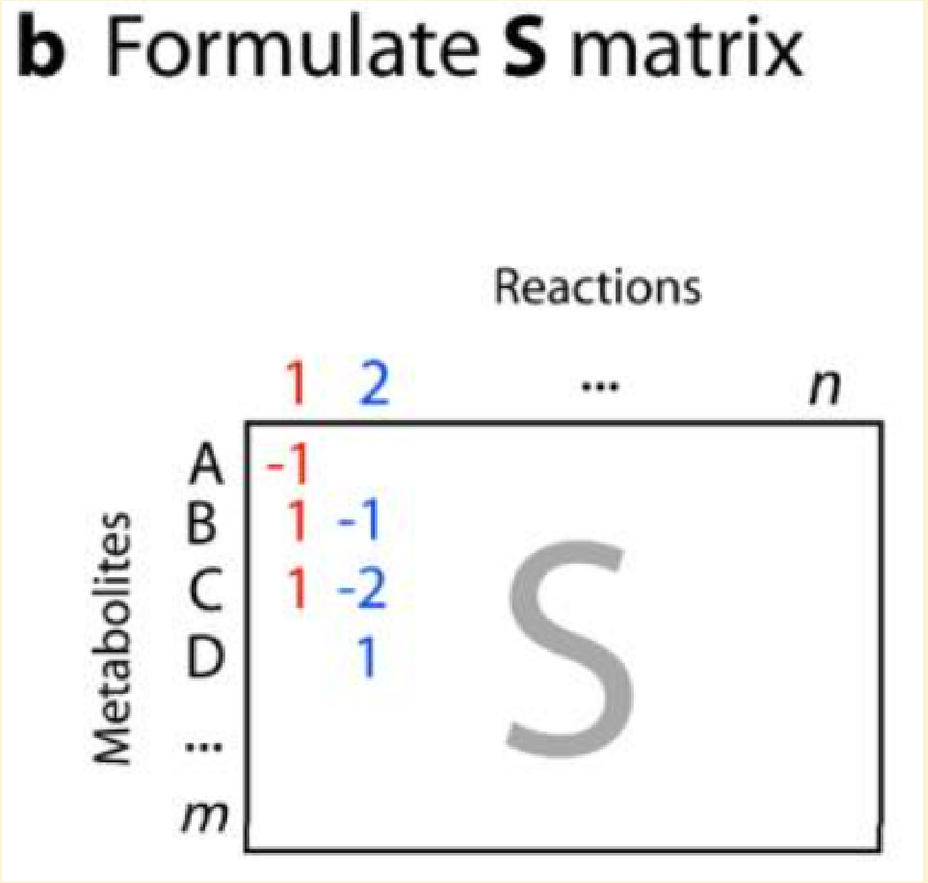
A metabolic network reconstruction is built, consisting of a list of stoichiometrically balanced biochemical reactions



BASIC STEPS FOLLOWED

Step 2

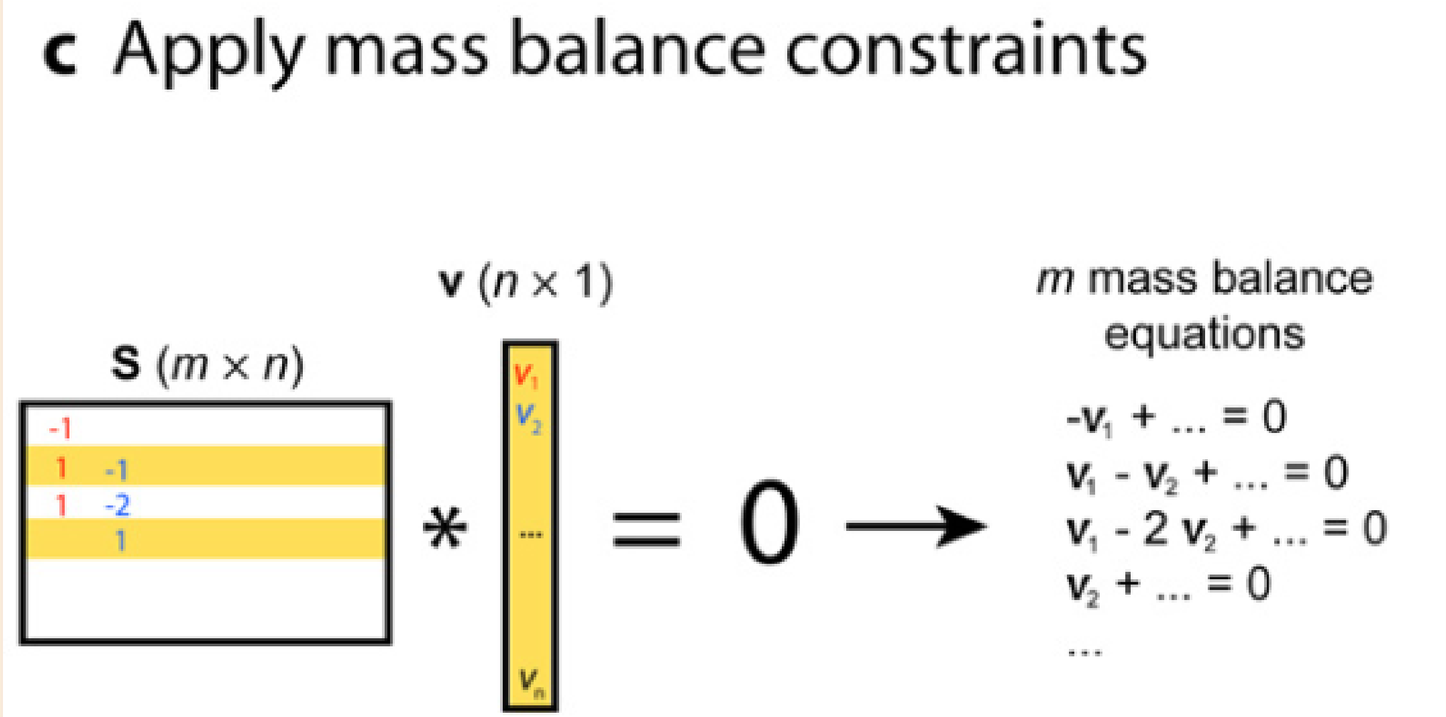
convert the reconstruction into a mathematical model by forming a matrix (labeled S) in which each row represents a metabolite and each column represents a reaction.



BASIC STEPS FOLLOWED

Step 3

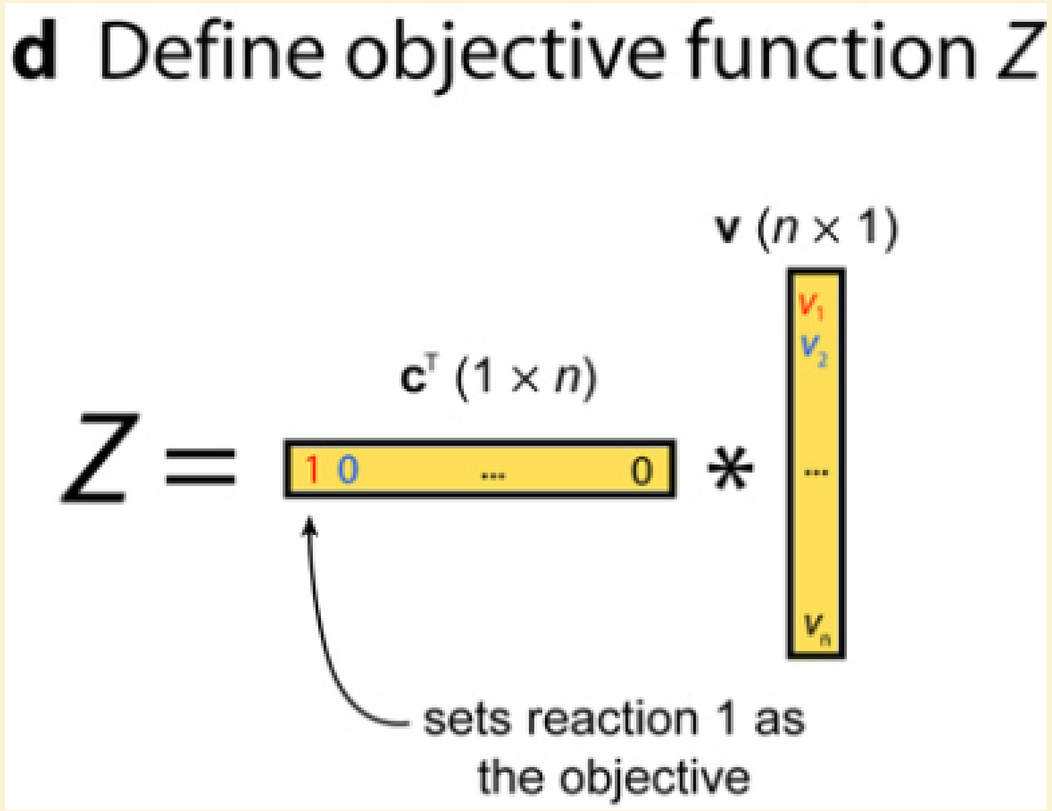
At steady state, the flux through each reaction is given by the equation Sv=0. Since there are more reactions than there are compounds, there is no unique solution to this system of equations.



BASIC STEPS FOLLOWED

Step 4

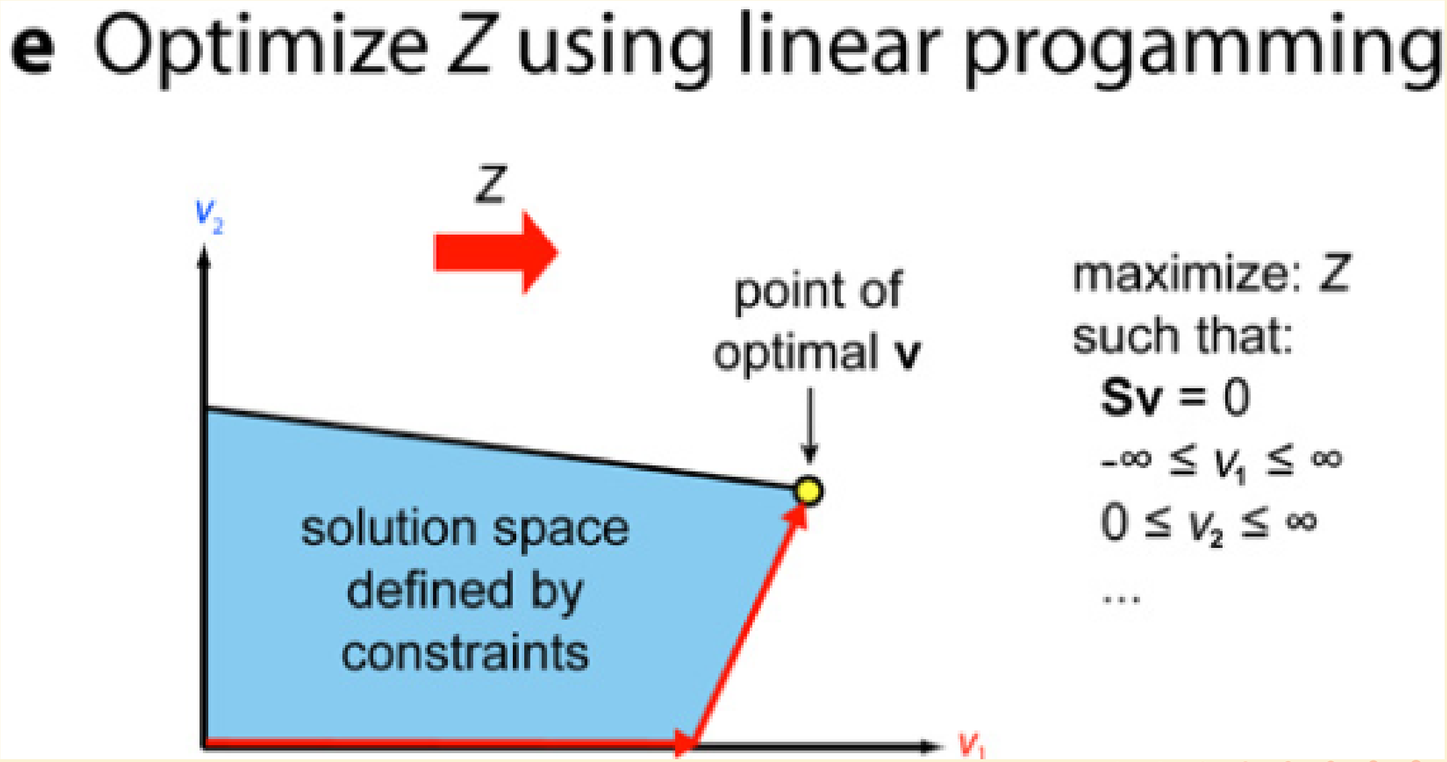
An objective function is defined as Z = cTv, where c is a vector of weights (indicating how much each reaction contributes to the objective function). only one reaction is desired for maximization or minimization, c is a vector of zeros with a one at the position of the reaction of interest



BASIC STEPS FOLLOWED

Step 5

Finally, linear programming can be used to identify a particular flux distribution that maximizes or minimizes this objective function while observing the constraints imposed by the mass balance equations and reaction bounds.



TOOLS FOR FBA

- FBA computations, which fall into the category of constraint-based reconstruction and analysis (COBRA) methods, can be performed using several available tools

- Models for the COBRA Toolbox are saved in the Systems Biology Markup Language (SBML)

We implemented the supplementary tutorial which allowed us to explore the library. The model that we used was different from the one used in the paper and contained more reactions. We also checked by how much our results changed due to this and found that it wasn't drastic.

ASSUMPTIONS & ISSUES

Major assumption:

The system of mass balance equations at steady state (dx/dt=0)

Limitations / Issues:

First

it cannot predict metabolite concentrations because it does not use kinetic parameters

Second

FBA does not account for regulatory effects. Which may lead to inaccurate predictions sometimes

MODIFIED FBA

- **Dynamic FBA**

  Predictive algorithm for time profiles of metabolism in the unsteady state of batch and fed batch culture.

- **Regulatory FBA**

  Accounts for the transitions in the utilization of metabolic pathway, such as change in acetate metabolism to acetate metabolism.

DYNAMIC FBA

Aim:

- The paper shows the FB model with an objectice of optimizing the growth rate is an appropriate representation of metabolism under the conditions of aerobic chemostat, batch, and fed-batch conditions as well as anaerobic batch condtions.

- They also show that time profiles for growth and by-product concentration in the culture media for batch and fed-batch cultures can be predicted using the inital conditions of the culture.

link to paper

CULTURE

- An E-coli K-12 strain, W3110 (ATCC 27325) was used for experiments. Strain is nearly wild type
- Defined M9 medium (Na_2HPO_4 [6 g/liter], KH_2PO_4 [3 g/liter], NaCl [0.5 g/liter], NH_4Cl [1 g/liter], $MgSO_4$ [2 mM], $CaCl_2$ [0.1 mM], $FeCl_3$ [0.01 mM]) was used for all the experiments with 2 g of glucose per liter except for the fed-batch experiments, in which glucose was continuously added
- A temperature of 38°C was maintained for the culture in the bioreactor as well as in the incubator

ITERATIVE ALGORITHM

1. The experimental time was divided into small time steps, Δt. The initial concentration values were specified.

2. Substrate concentration (Sc) (millimoles per liter) is determined from the substrate concentration predicted for the previous step (Sco) plus any additional substrate provided in a fed-batch mode:

$$S_c = S_{co} + \frac{\text{supply} \cdot \Delta t}{\text{volume}}$$

ITERATIVE ALGORITHM

3. The substrate concentration is appropriately scaled to define the amount of substrate available per unit of biomass per unit of time (millimoles per gram [dry weight] per hour) where X is cell density:

$$\text{Substrate available} = \frac{S_c}{X \cdot \Delta t}$$

4. The flux balance model is used to evaluate the actual substrate uptake (Su) (this may be less than the amount of substrate available), the growth rate (μ), and potential by-product secretion.

ITERATIVE ALGORITHM

5. Concentrations for the next time step are calculated from the standard differential equations:

$$\frac{dX}{dt} = \mu X \rightarrow X = X_o \cdot e^{\mu \Delta t}$$

$$\frac{\partial S_c}{\partial t} = -S_u \cdot X \rightarrow S_c = S_{co} + \frac{S_u}{\mu} X_o (1 - e^{\mu \Delta t})$$

RESULTS

To apply the FB model the strain specific parameters (finite uper limit on rate of metabolism per unit (dry wt) of bacteria, maximum enzymatic limits of oxygen and glucose uptake) first need to be determined. In the paper they have derived this from batch experiments. Biomass scaling and maintenance requirments have also been derived.

DOUBT

To determine the maintenance requirments they have plotted glucose uptake as a function of the growth rate in the chemostat. They specified that non growth associated maintenance was calculated using the y intercept of lower slope and growth associated maintenance using upper but why?

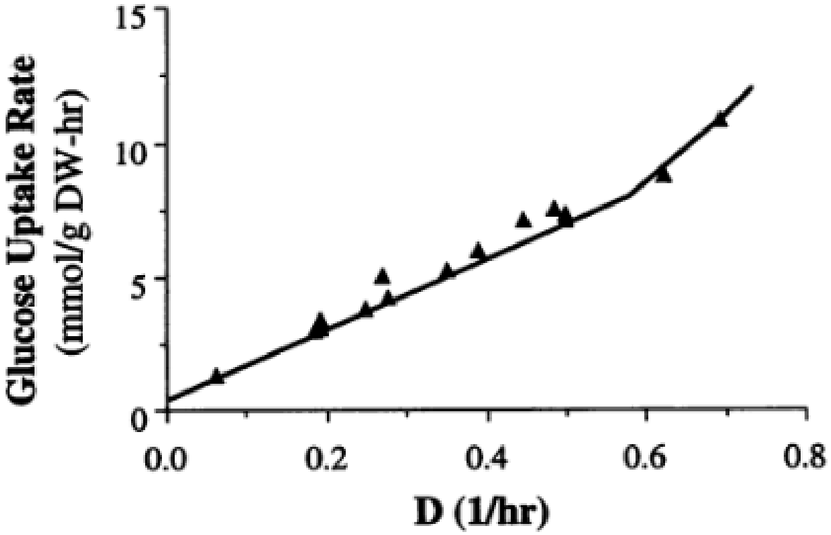


FIG. 4. Determination of biomass and maintenance requirements on the basis of chemostat experiments. There were no mineral limitations in the chemostat. The solid line denotes the best fit of data from the flux balance model to the experimental datum points shown. The non-growth-associated maintenance requirement determined from the y-intercept is 7.6 mmol of ATP per g (dry weight [DW]) per h, while the growth-associated maintenance requirement is defined as 13 mmol of ATP per g (dry weight). In addition, biomass requirements are scaled to be 30% higher than the defined values reported previously (5) to account for strain-specific differences. D, dilution rate in the chemostat.

AEROBIC BATCH CULTURE

- The flux balance model was able to accurately predict time profiles for the cell density as well as the glucose uptake rate. The model was also able to predict acetate secretion and accumulation in the medium.

- The model accurately predicted the reutilization of acetate, but it was unable to quantitatively account for the time required to make the transition from glucose consumption to acetate consumption.

- Transitions between different pathway utilizations are a function of genetic regulatory processes. The flux balance model incorporates only metabolic pathway information and thus cannot predict the time course of genetic regulation.

AEROBIC FED-BATCH CULTURE

High cell density and relatively low glucose feed rate:

- The model accurately predicted the time profile of cell density and acetate concentration, and it also showed no accumulation of glucose in the medium, consistent with experimental observations.

Higher glucose feed rate relative to cell density :

- In the early phase of this culture, acetate was observed to accumulate, followed by its reconsumption later in the culture.
- In the later part of the culture glucose and acetate were metabolized simulaneously. (an interesting phenomena)

AEROBIC FED-BATCH CULTURE

Quite low Inoculum density :

- **Correctly predicted in this case as well**
- **Glucose accumulates at low cell density as a result of excess glucose feed as compared to the enzymatic uptake limit of cells**
- **Later in the culture as cell density increased the cells were able to consume all the glucose supplied**
- **Accumulation of acetate was observed. As cell densty increased and glucose was depleted the acetate accumlated was metabolized along with continuously fed glucose.**

ANAEROBIC BATCH CULTURE

- There was a high correlation between the model predictions and experimental data obtained.
- The 3 major by-products :- acetate, ethanol and formate were secreted and accumulated in the medium.
- The by-product succinate was experimentally observed but the model couldn't predict its formation
- There is a small deviation from optimal metabolism under anaerobic condtions.

Doubt: Why in the case of anaerobic conditions the model did not predict the product formation?

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THANK YOU