BBSRC

EPSRC

A genome scale model of *Cupriavidus necator* for platform chemical production

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Synthetic Biology Research Centre

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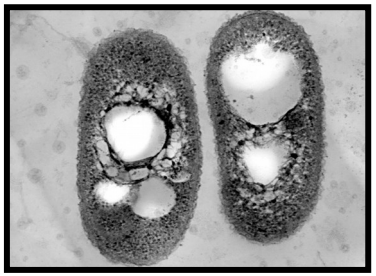
Waste into wealth using bacteria

Industrial waste

$CO_2 + H_2$

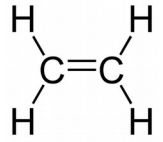
Microbial Fermentation



Cupriavidus necator

Flux balance analysis

ETHYLENE



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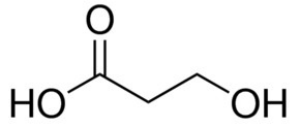

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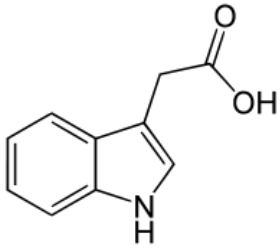
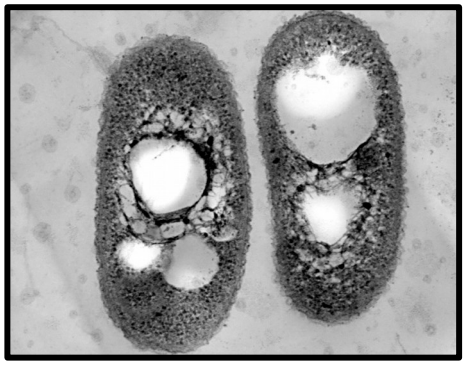


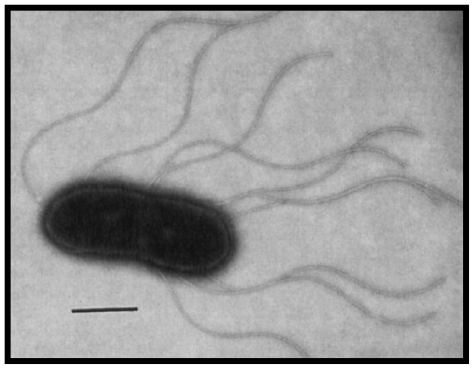
Genome scale model of *Cupriavidus necator*

C. necator H16 an ideal chassis for biotechnology

- Grows on organic substrates or **H_2** and **CO_2** under aerobic conditions
- Grows to high-cell densities under lithoautotrophic or heterotrophic conditions
- Produces large amounts of a biodegradable polymer polyhydroxybutyrate (PHB)



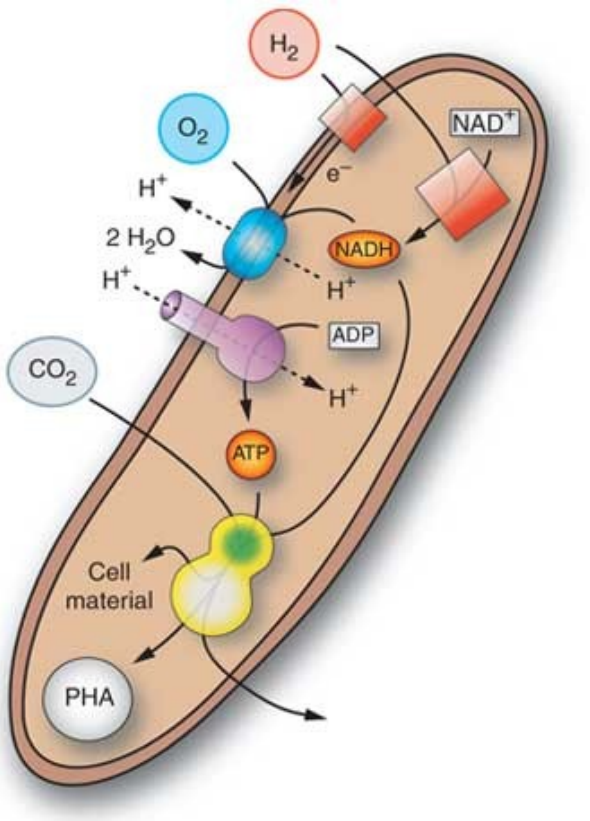
STEM picture of *Cupriavidus necator* harbouring PHB granules



Flagellation of strain N-1. Bar, 1.0 µm

C. necator lithoautotrophic metabolism

- Carbon dioxide is fixed via the Calvin cycle
- Membrane bound hydrogenase directly connected to the electron transport chain (ETC) for generating ATP
- Soluble hydrogenase that is coupled to NADH synthesis that is required for the Calvin cycle or ETC
- Oxygen final electron acceptor (under anaerobic conditions nitrate is used)



Lithoautotrophic metabolism

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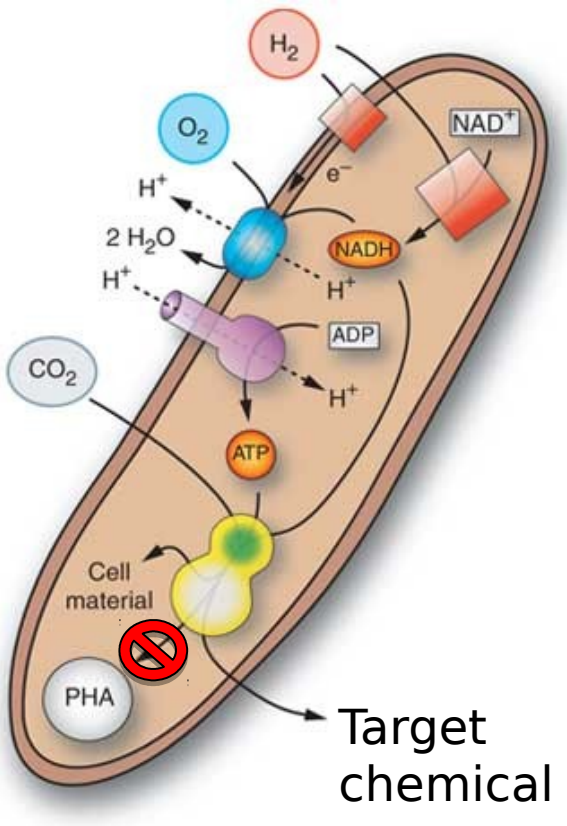


Lithoautotrophic metabolism

Genome scale model of *C. necator*

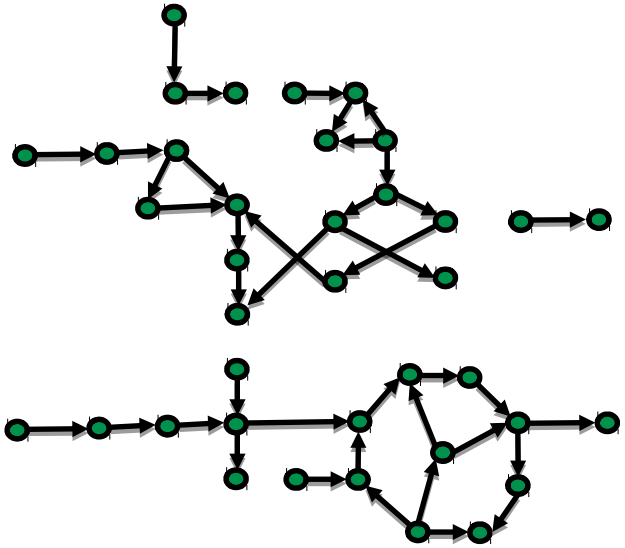
- Constructed genome scale model of *C. necator* using Cell Systems Modelling Group pipeline

BioCyc
Database Collection

$A \longrightarrow B + C$

$B + 2\,C \longrightarrow D$

$2\,H + 3\,C \longrightarrow G$



Genome scale model of *C. necator*

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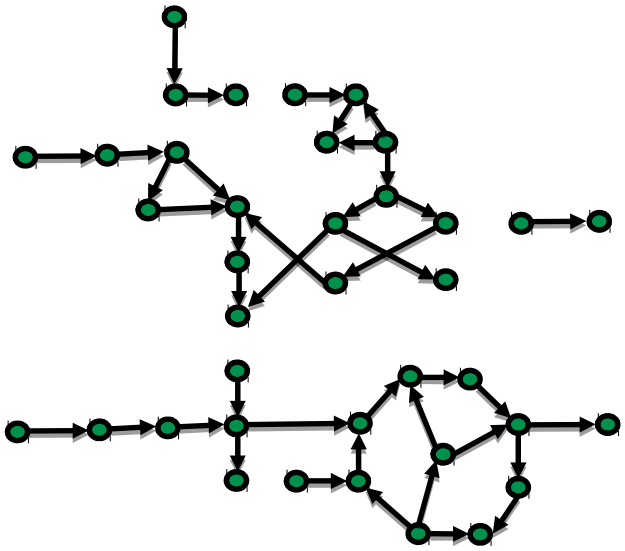
  - 912 BioCyc reactions (AutoReutro.spy)



Genome scale model of *C. necator*

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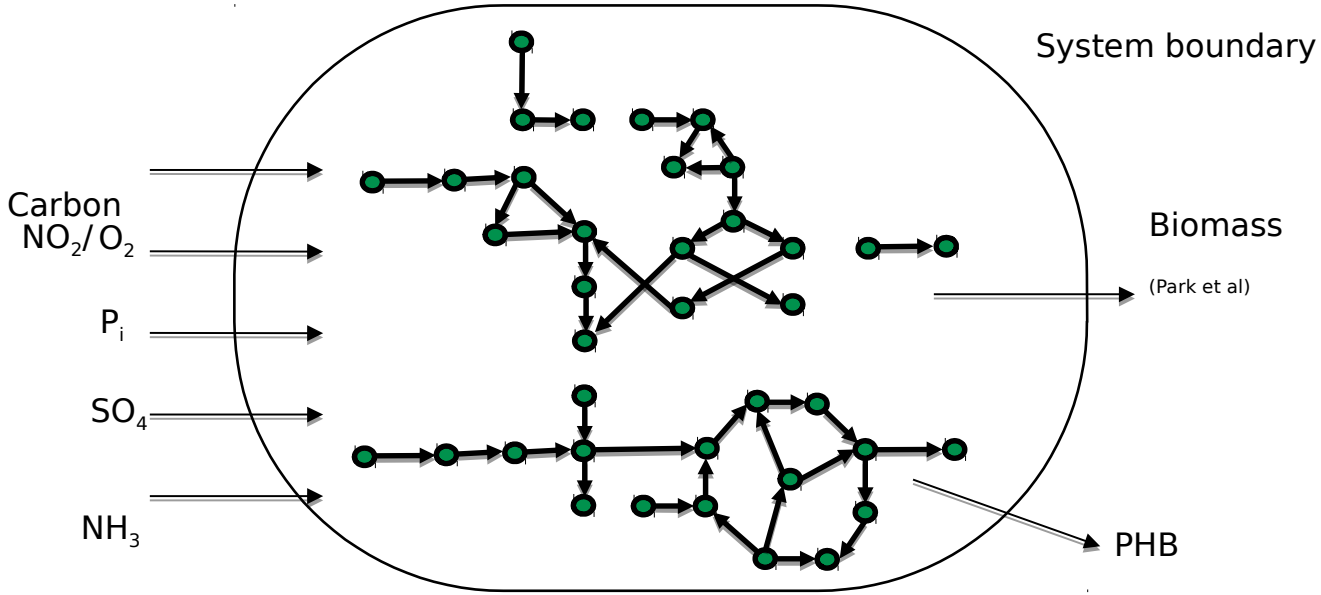
  - 97 Transport reactions (Transporters.spy)



Genome scale model of *C. necator*

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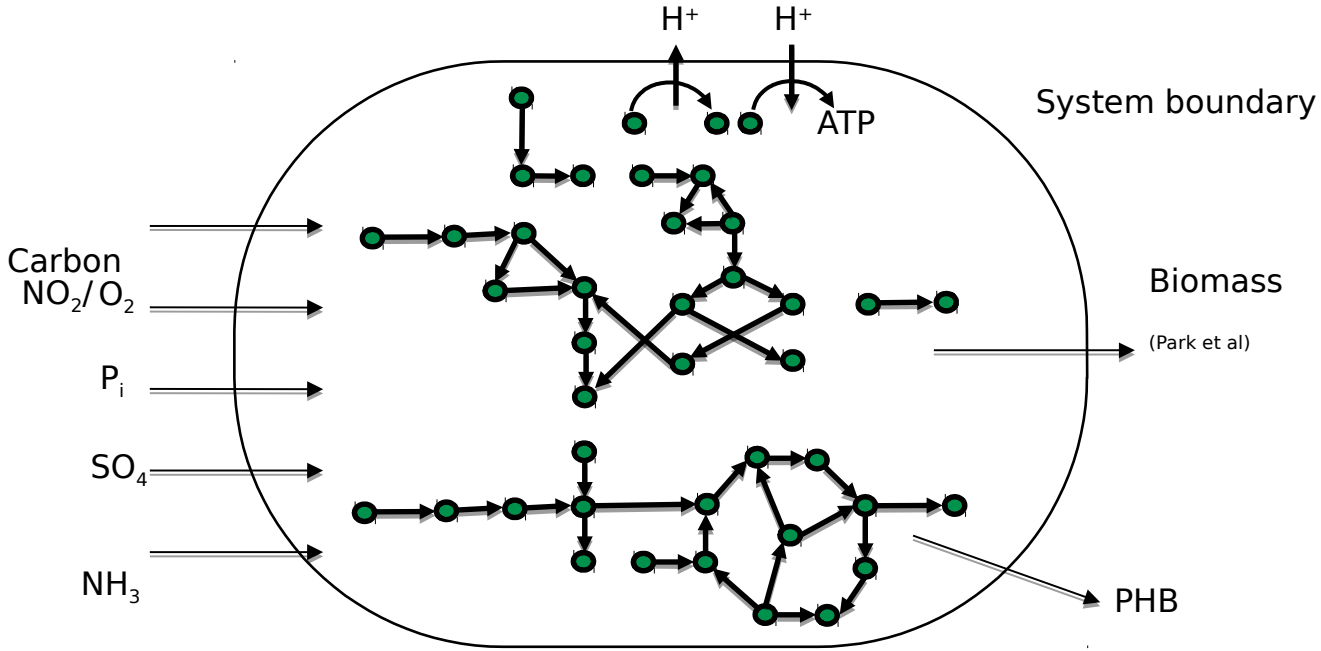
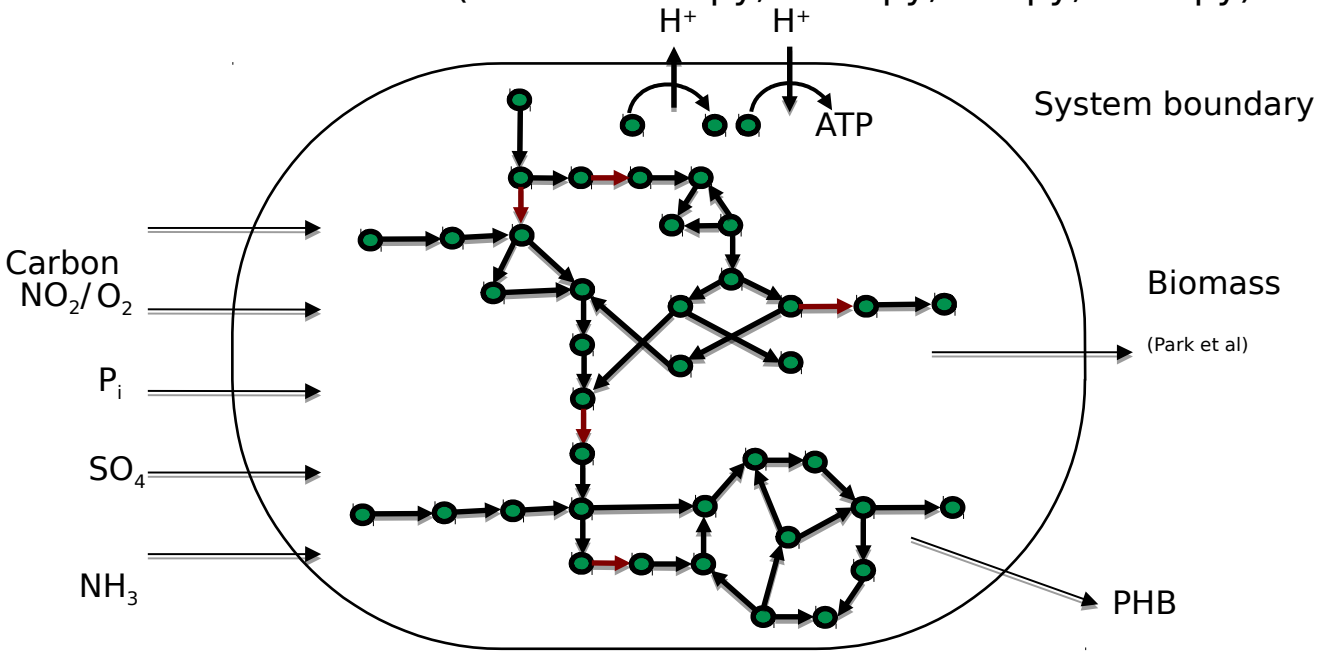
  - 912 BioCyc reactions (AutroReutro.spy)
  - 97 Transport reactions (Transporters.spy)
  - 16 Electron transport chain (ETC.spy)

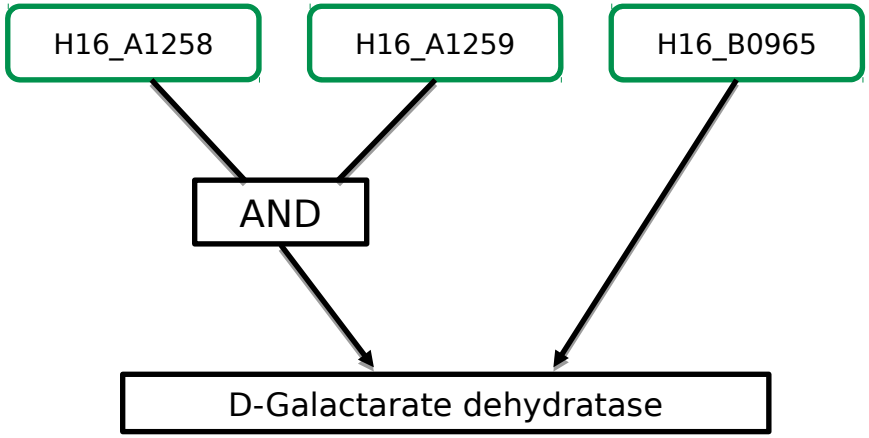


Genome scale model of *C. necator*

- Constructed genome scale model of *C. necator* using Cell Systems Modelling Group pipeline
  - 912 BioCyc reactions (AutroReutro.spy)
  - 97 Transport reactions (Transporters.spy)
  - 16 Electron transport chain (ETC.spy)
  - 260 Additional reactions (ExtraReacs.spy, PHL.spy, FA.spy, LPS.spy)



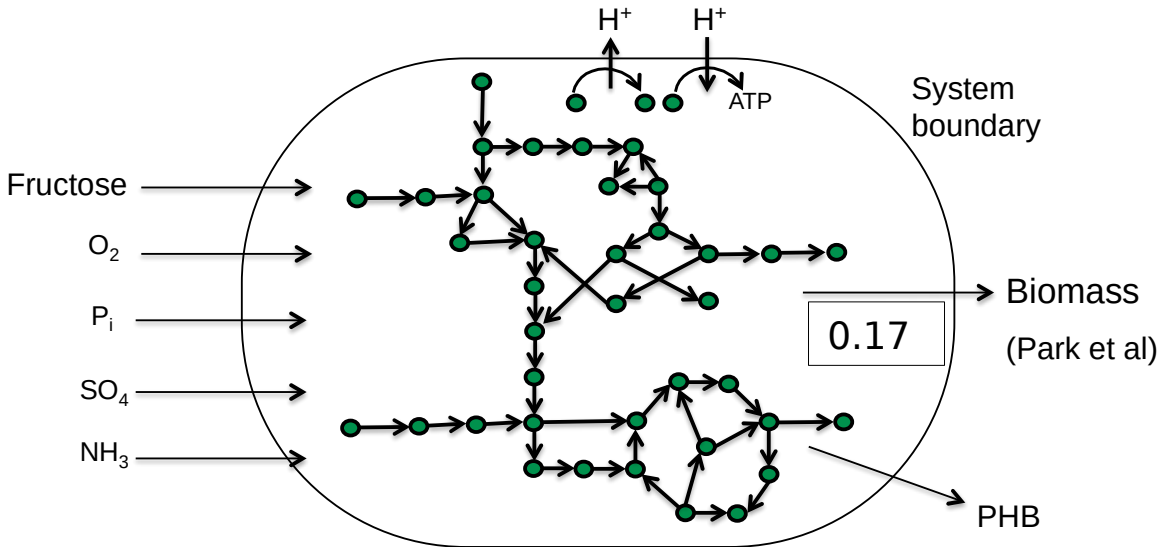
Gene-reaction associations



Galactarate dehydratase rxn : (H16_A1258 AND H16_A1259) OR H16_B0965

- Further network curation
- Carry out gene knockout analysis
- Integrate gene expression data

Genome scale model validation

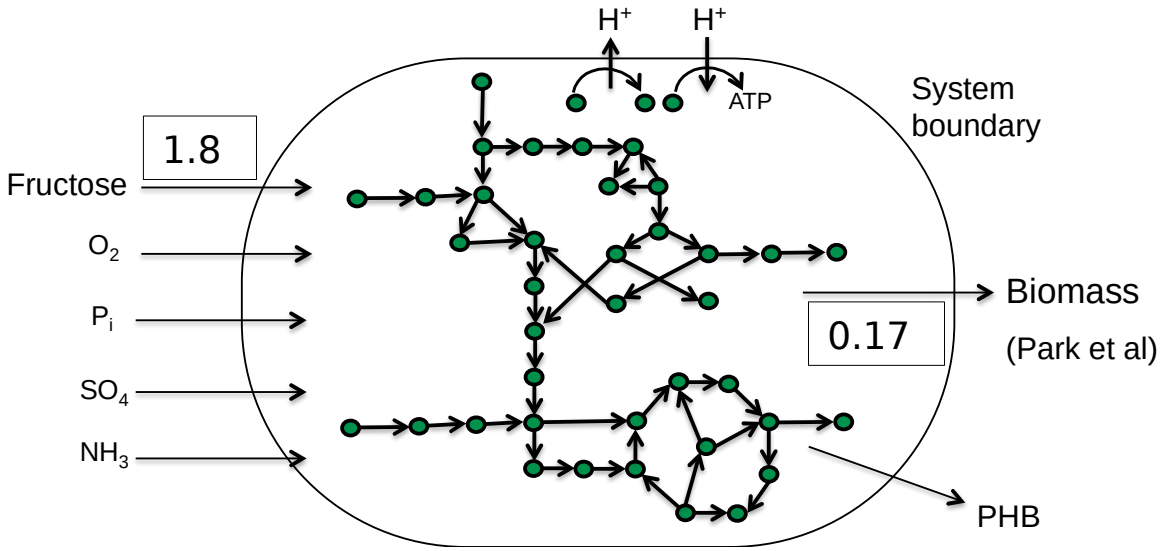


Substrate (mmol gDCW h)	Growth rate (1/h)
Fructose (2.1 ± 0.3)	0.17 ± 0.03

$$\text{minimize} : |\mathbf{v}|$$

$$\text{subject to} : \begin{cases} Nv = 0 \\ v_{\text{bio}} = 0.17 \\ v_{\text{ATPase}} = J_{\text{ATPase}} \end{cases}$$

Genome scale model validation



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Genome scale model validation – ammonia limitation

$$\text{minimize} : |\mathbf{v}|$$

← Objective: min. sum of fluxes

$$\text{subject to} \begin{cases} Nv = 0 \\ v_{\mathrm{NH_4}} = 1.0 \\ v_{\mathrm{biomass}} = 0.1 \\ v_{\mathrm{fru}} = x \end{cases}$$

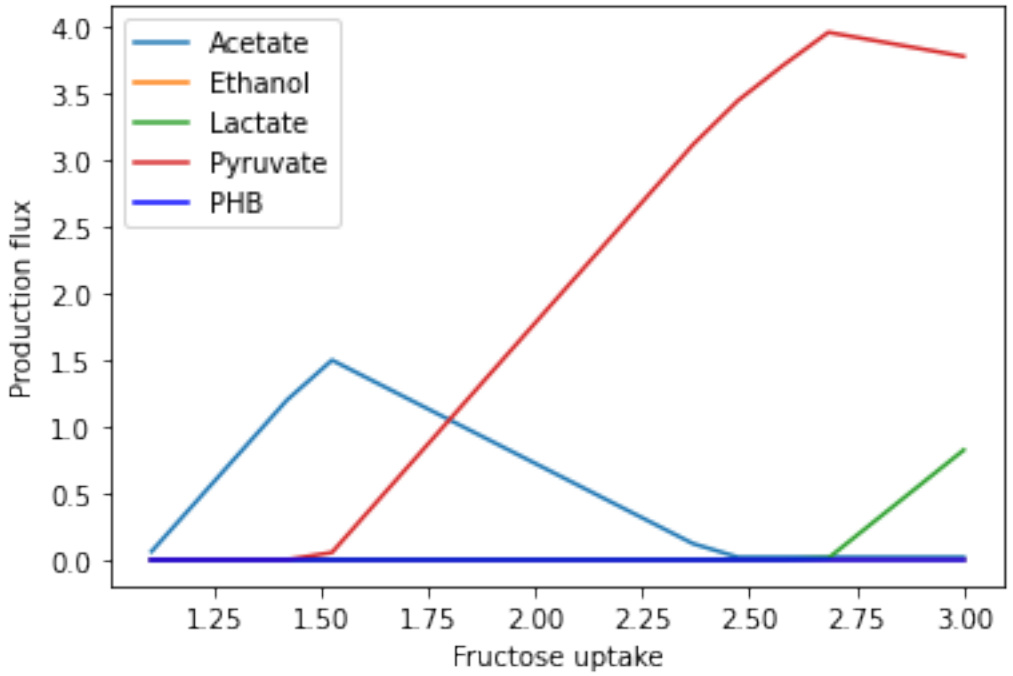
← Steady state constraint

← Fixed ammonium uptake

← Fixed biomass

← Fructose uptake, varied between 1 and 3

Genome scale model validation – ammonia limitation



Genome scale model validation – ammonia limitation

Integrated gene expression data using iMAT approach to reduce the solution space

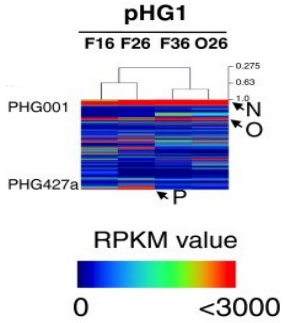
Divide genes into 3 levels: highly, moderately and lowly expressed (1, 0, -1)

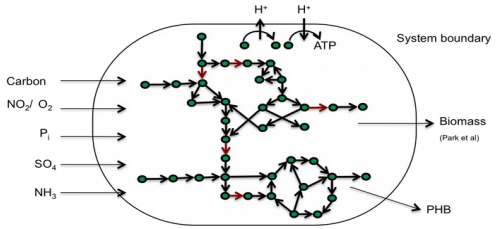
↓

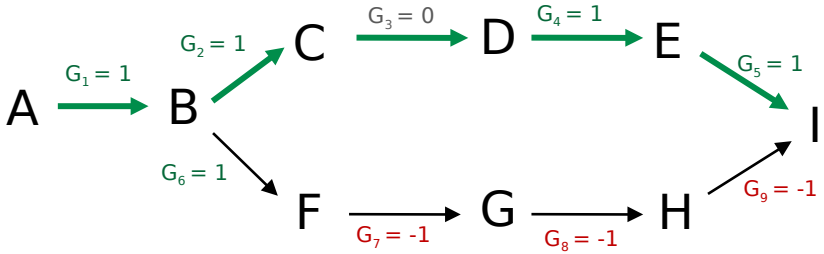
Integrate using iMAT algorithm – mixed integer linear program that maximises consistency to the gene expression level

↓

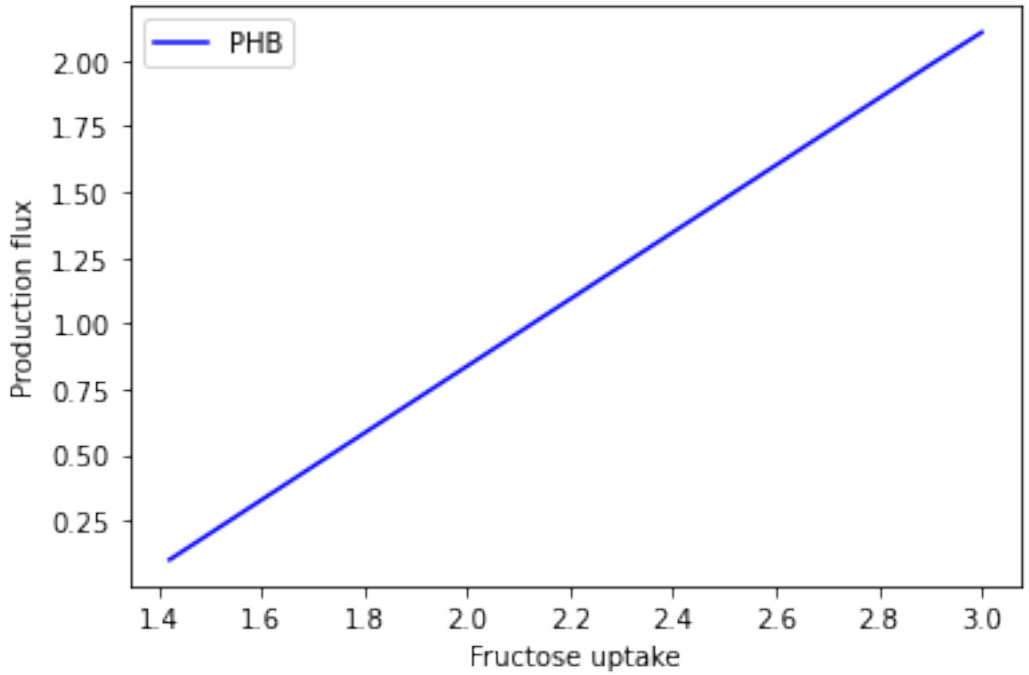
Generation of condition-specific model with reduced number of reactions





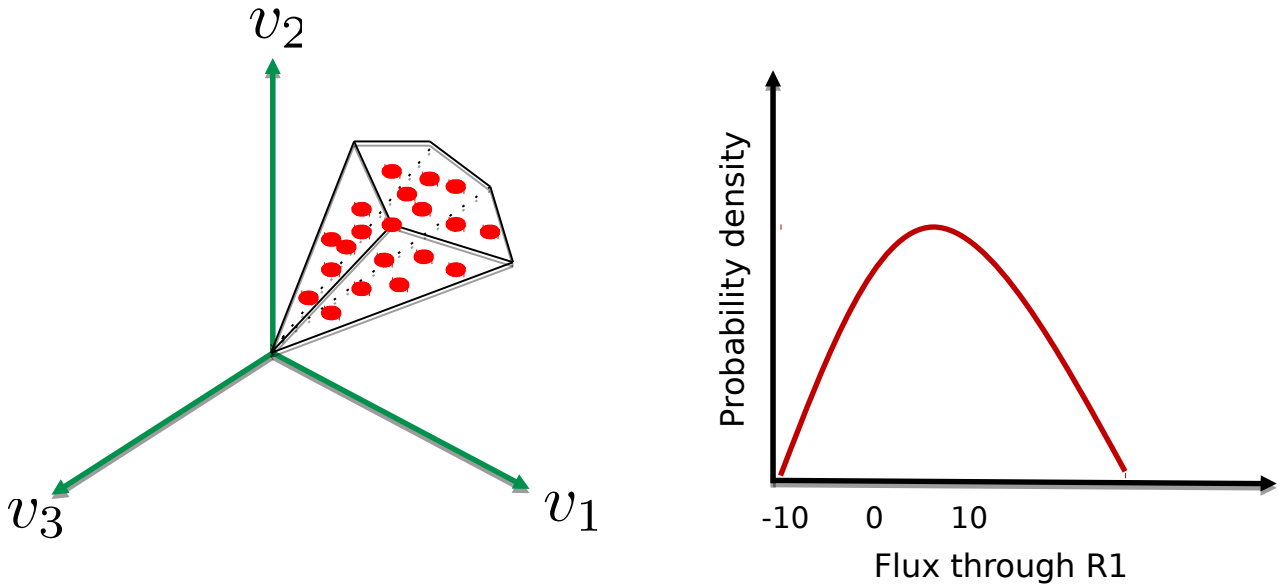


Genome scale model validation – ammonia limitation



Genome scale model validation – ammonia limitation

Flux sampling analysis scans the solution space to provide distribution of flux values per reaction. Compare flux distributions across two different conditions to find those that are differentially altered



The samples must be a ‘good’ representation of the solution space.

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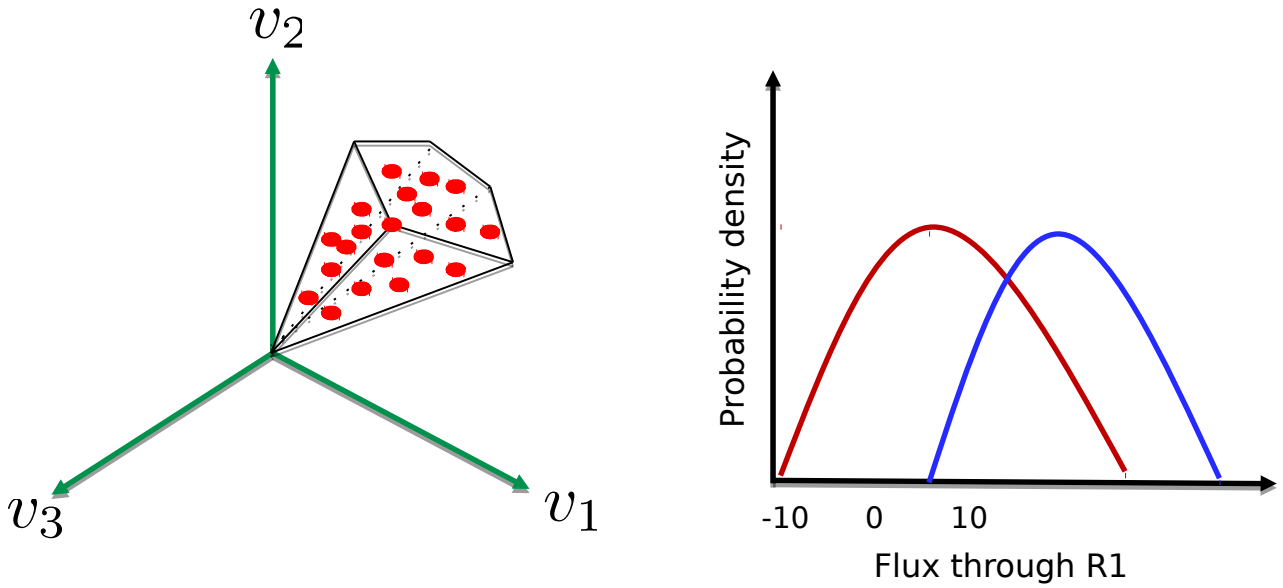
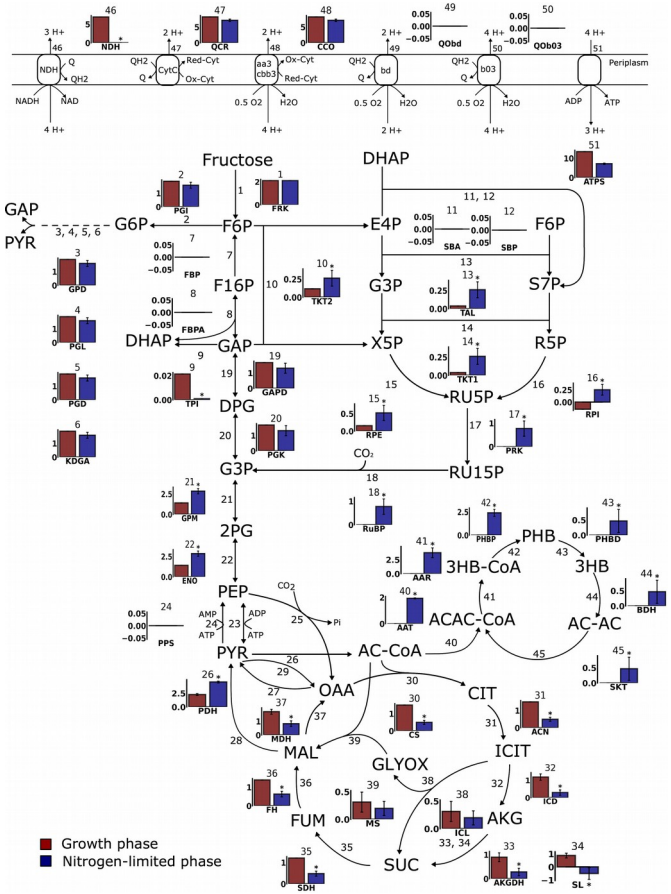


The samples must be a ‘good’ representation of the solution space.

Genome scale model validation – ammonia limitation

- The results show the mean flux value for the growth phase predicted fluxes and the nitrogen-limited phase predicted fluxes
- PHB production and degradation is active
- Calvin-Benson cycle is active in ammonia limited conditions
- TCA cycle flux decreases in ammonia limited conditions



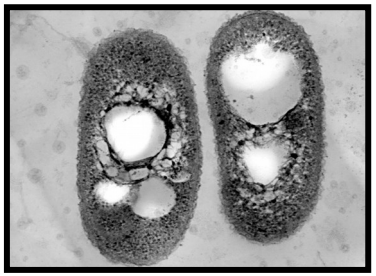
Redirecting flux towards platform chemicals

Waste into wealth using bacteria

Industrial waste

$CO_2 + H_2$

Microbial Fermentation



Cupriavidus necator

Flux balance analysis

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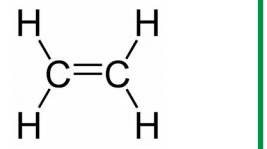

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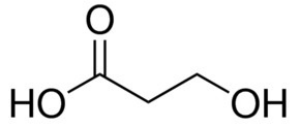

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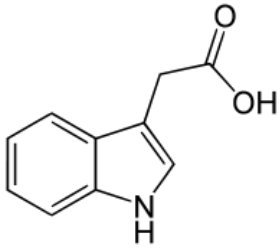


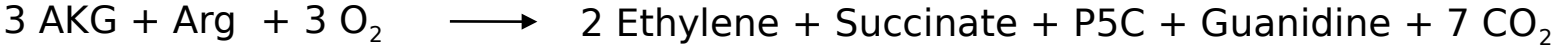
Ethylene production

Widely used in the chemical industry, worldwide production exceeds **150 million tons**

Currently produced from **steam cracking**, which releases vast quantities of **CO_2**

Already produced in microorganisms that contain the **ethylene forming enzyme (EFE)** but with low yields

EFE stoichiometry:



Maximum theoretical yields of ethylene

$$\max.\ v_{\text{ethylene}}$$

$$\text{subject to}: \begin{cases} Nv = 0 \\ 0 \leq v_{\text{fru}} \leq 2 \\ 0.05 \leq v_{\text{bio}} \leq \infty \end{cases}$$

Set objective function to maximise flux to ethylene transporter

Maximum theoretical yields of ethylene

$$\max.\ v_{\text{ethylene}}$$

$$\text{subject to}: \begin{cases} Nv = 0 \\ 0 \leq v_{\text{fru}} \leq 2 \\ 0.05 \leq v_{\text{bio}} \leq \infty \end{cases}$$

Steady state constraint

Maximum theoretical yields of ethylene

$$\max.\ v_{\text{ethylene}}$$

$$\text{subject to}: \begin{cases} Nv = 0 \\ 0 \leq v_{\text{fru}} \leq 2 \\ 0.05 \leq v_{\text{bio}} \leq \infty \end{cases}$$

Constrain the fructose uptake rate to be less or equal to 2 mmol $gDCW^{-1}h^{-1}$

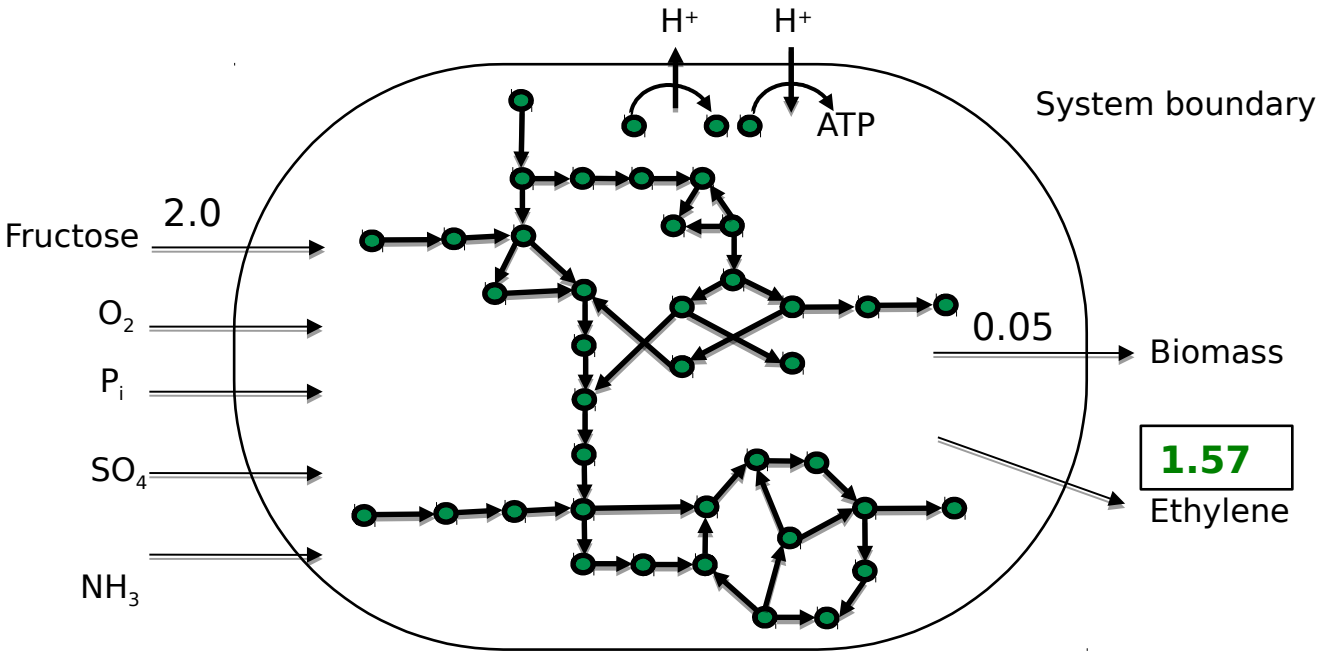
Maximum theoretical yields of ethylene

$$\max.\ v_{\text{ethylene}}$$

$$\text{subject to}: \begin{cases} Nv = 0 \\ 0 \leq v_{\text{fru}} \leq 2 \\ 0.05 \leq v_{\text{bio}} \leq \infty \end{cases}$$

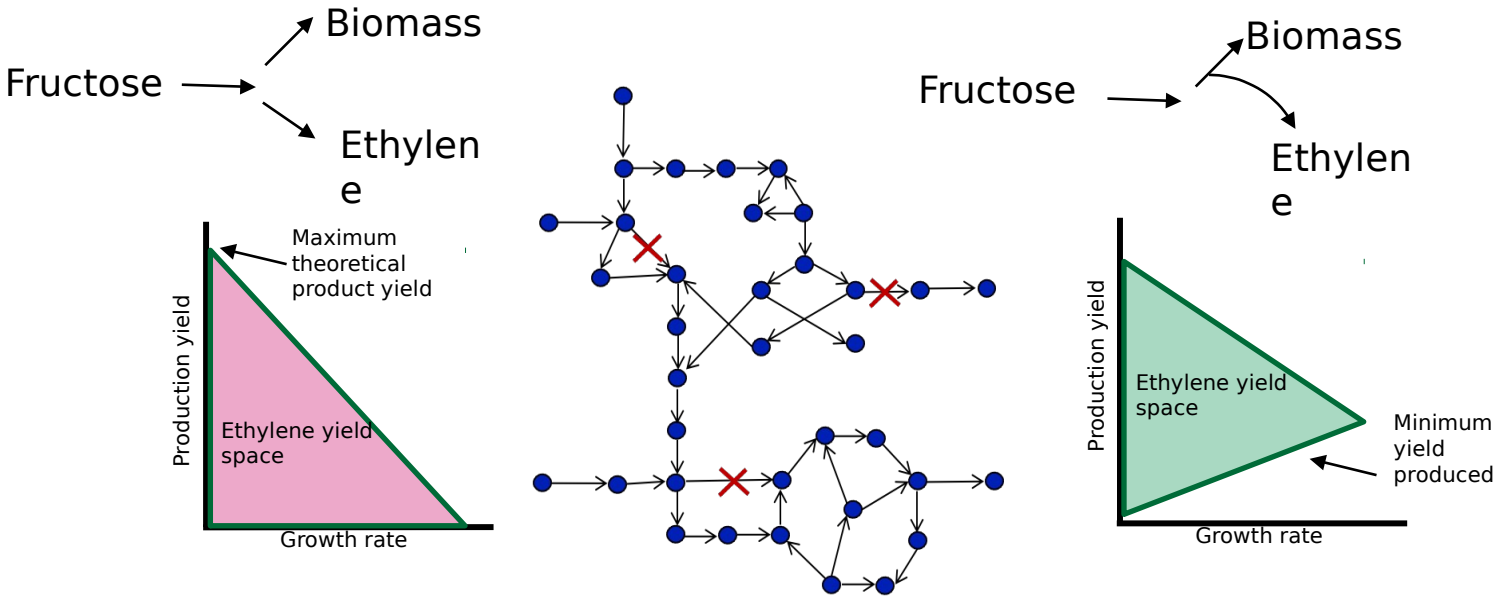
Constrain biomass to 0.05 h^{-1} or higher.

Maximum theoretical yields of ethylene



All rates in:
mmol gDCW^{-1} h^{-1}

Redirecting flux towards ethylene via growth coupling



Redirecting flux towards ethylene via growth coupling

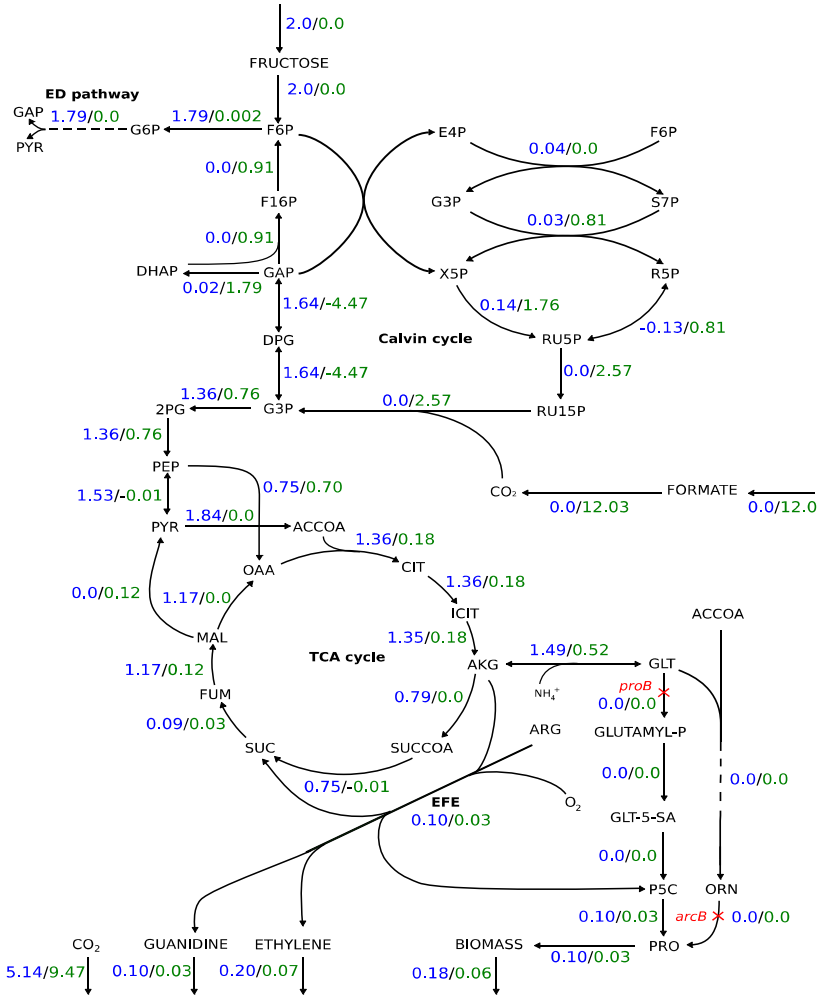
Simulated double reaction knockouts in the model with maximisation of biomass as objective function

$$\max.\ v_{\text{Biomass}}$$

$$\text{subject to}: \begin{cases} Nv = 0 \\ 0 \leq v_{\text{fru}} \leq 2 \\ v_i = 0 \\ v_j = 0 \end{cases}$$

Redirecting flux towards ethylene via growth coupling

- Identified solution that blocks proline biosynthesis by KO of 2 reactions
- EFE becomes essential for restoring proline biosynthesis



Conclusions

- GSM predicts growth rates with high accuracy during growth phase
- Predicting ammonia limited conditions however has more variability in the model. Integrating OMICs data can reduce the variability and correctly produced PHB as sole product
- GSM is useful for predicting capabilities for producing non-native products and to assess and predict new engineering strategies
- Optimisation approaches can be used such as optGene, optKnock, optStrain to predict growth coupling strategies

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