



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

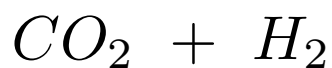
Nicole Pearcy

Synthetic Biology Research Centre

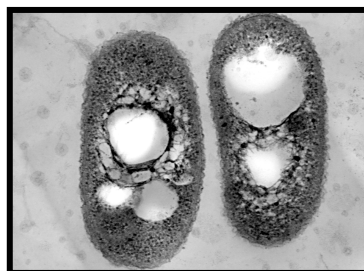
18th January, 2018

Waste into wealth using bacteria

Industrial waste

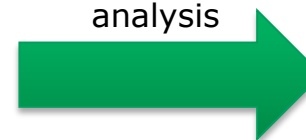


Microbial
 Fermentation

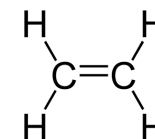


*Cupriavidus
 necator*

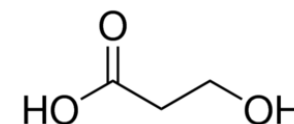
Flux balance
 analysis



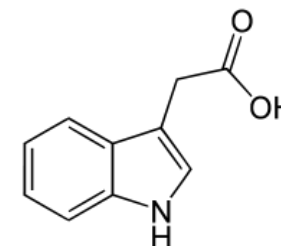
ETHYLENE



3-HYDROXY-PROPRIONATE

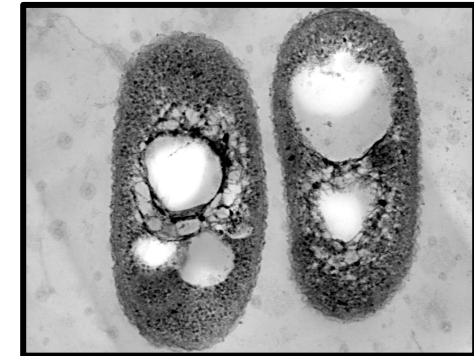


INDOLE-3-ACETATE

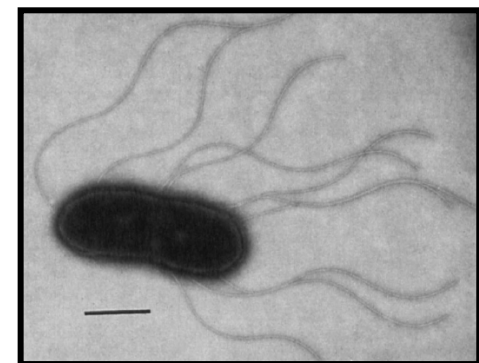


C. necator H16 an ideal chassis for biotechnology

- Facultatively **chemolithoautotrophic** bacteria - grow with organic substrates or H_2 and CO_2 under aerobic conditions
- Grow to high-cell densities under lithoautotrophic or heterotrophic conditions
- Produces large amounts of a biodegradable polymer polyhydroxybutyrate (PHB)



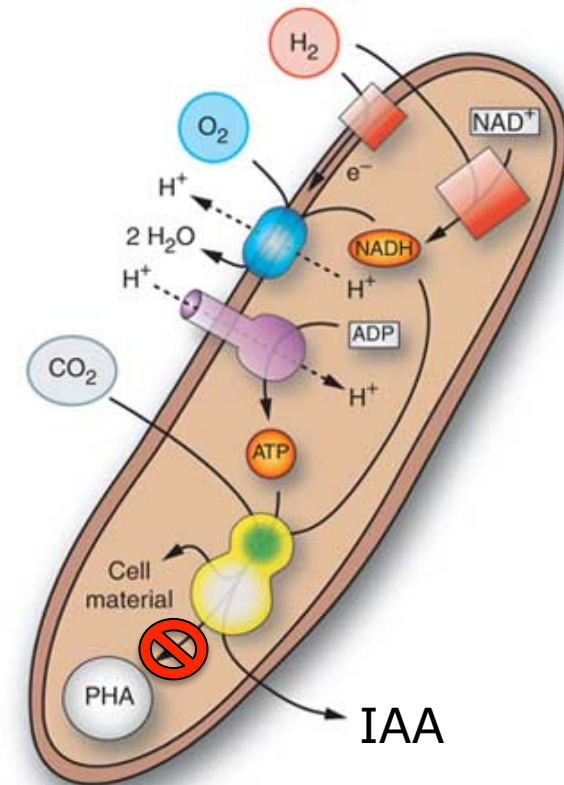
Flagellation of strain N-1.
Bar, 1.0 μm



STEM picture of *Cupriavidus necator* harbouring PHB granules

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)

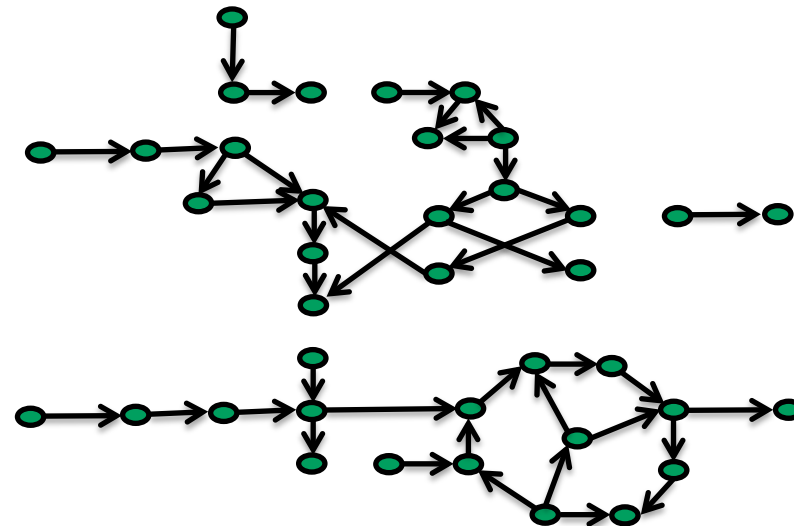
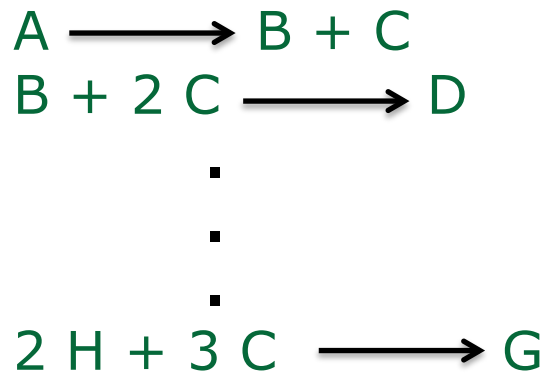


Lithoautotrophic metabolism

Genome scale model of *Cupriavidus necator*

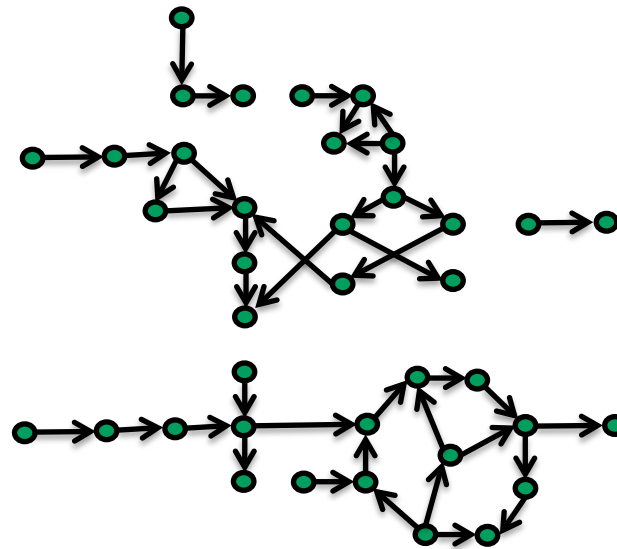
Genome scale model of *C. necator*

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



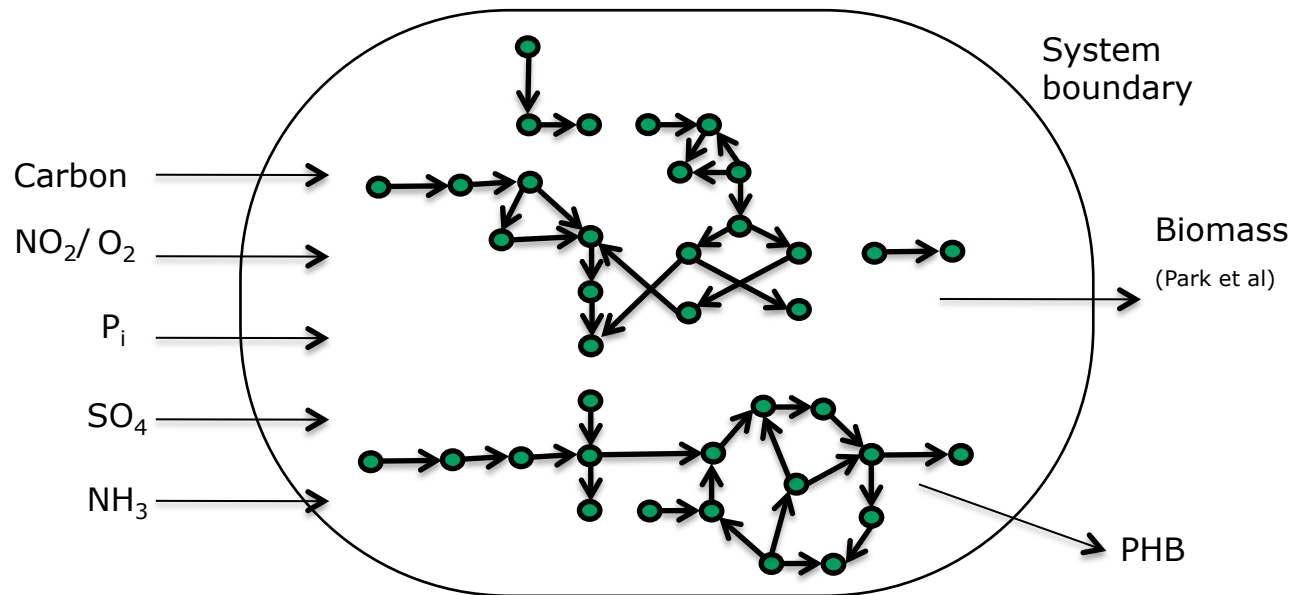
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 - BioCyc reactions (994)



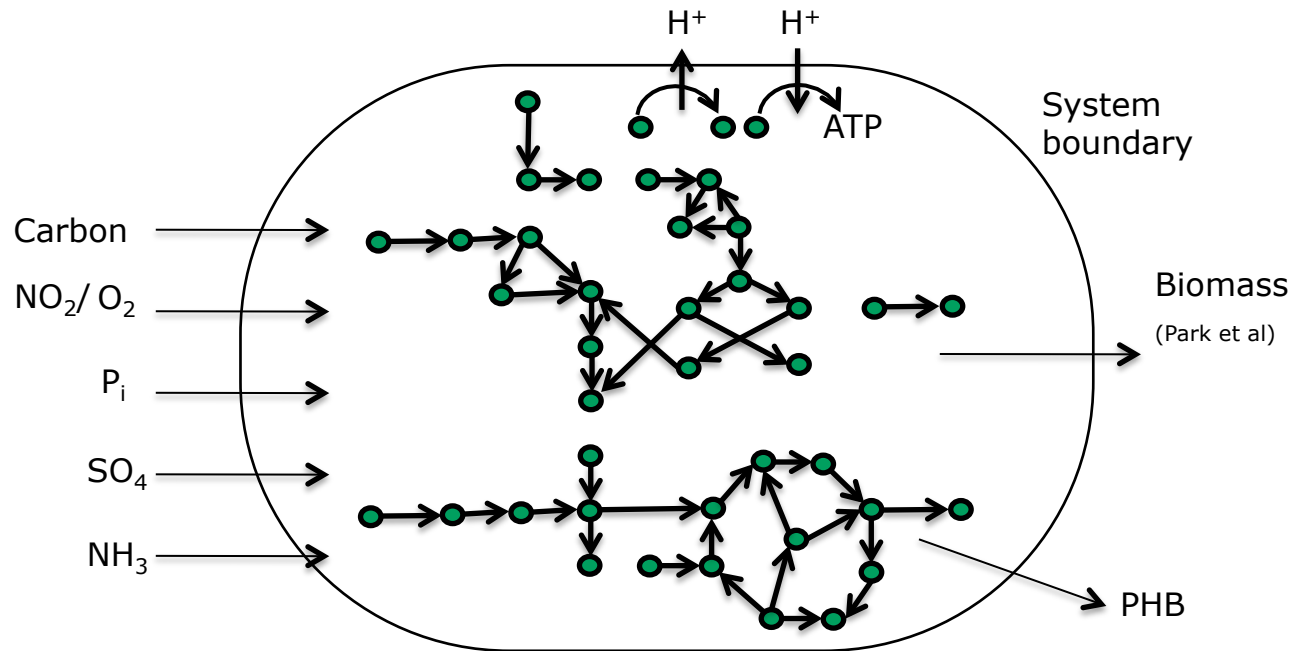
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 - Transport reactions (114)



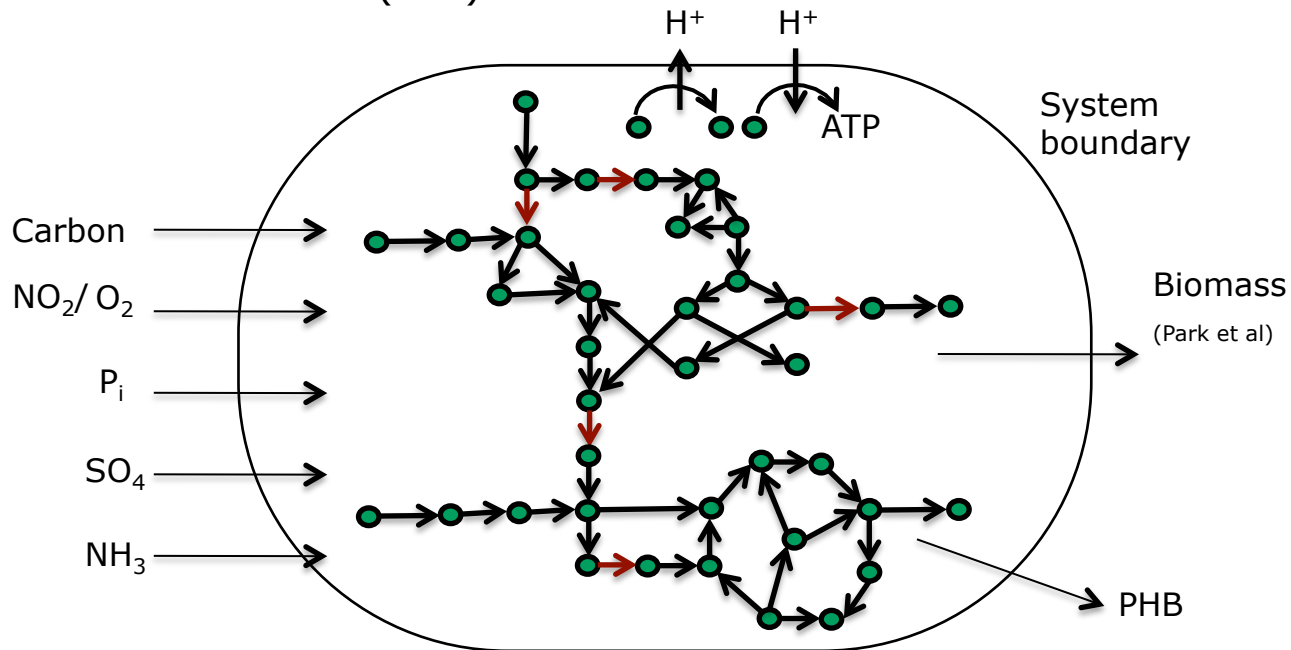
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 - BioCyc reactions (994)
 - Transport reactions (114)
 - Electron transport chain (15)
 - Additional reactions (113)

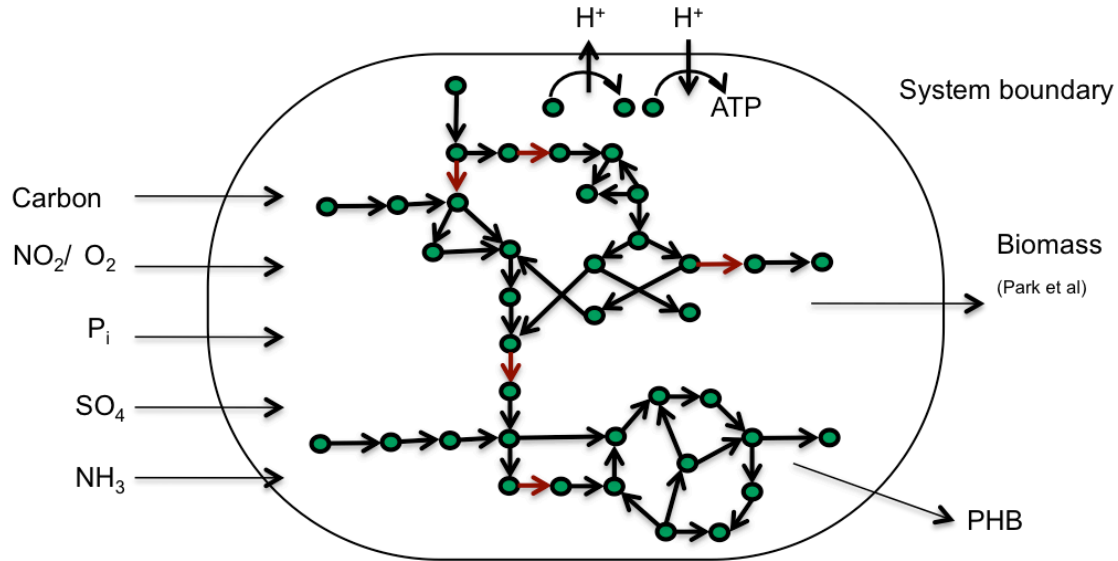


Genome scale model theoretical validation

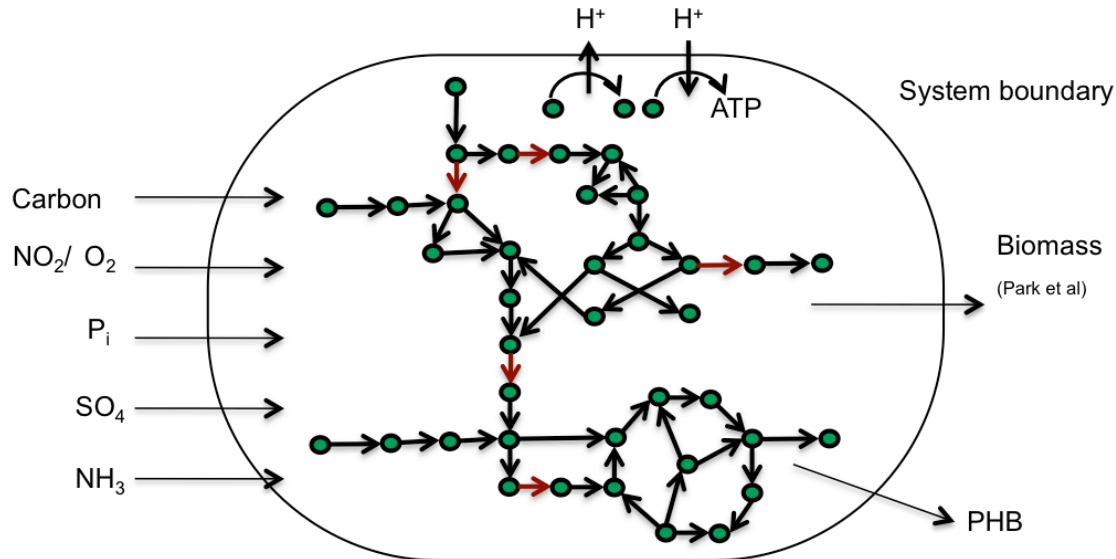
- Inconsistent stoichiometries (e.g. reactions producing carbon from nothing)
- Inconsistent subsets (checking irreversibility)
- Violations of conservation energy (making ATP from nothing)
- Iterative process, have to carry out inconsistency tests every time new reactions are added.

Takes around 2-3 months for theoretically validated model

Genome scale model validation

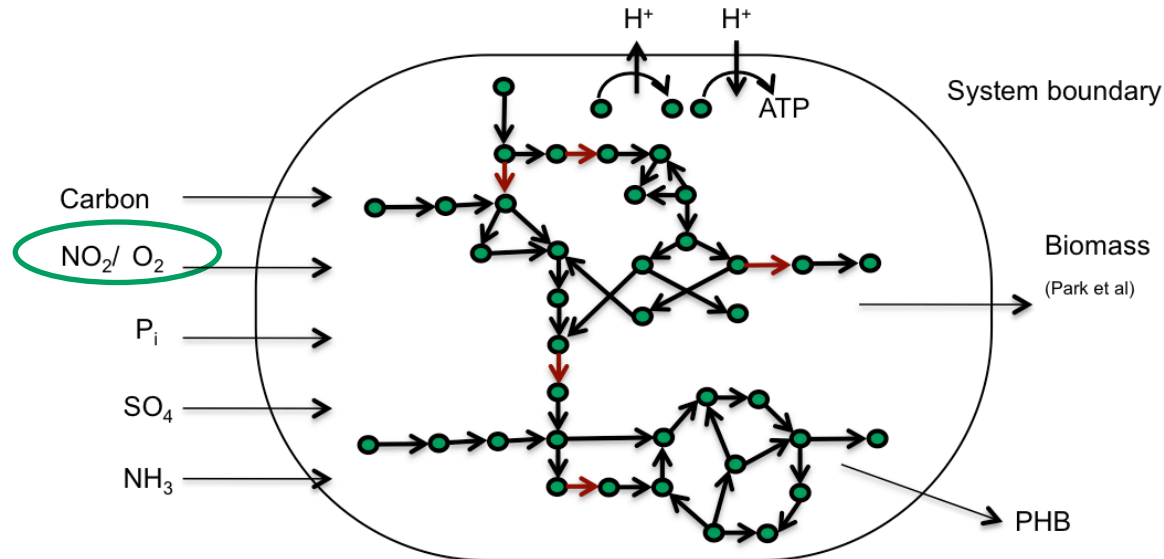


Genome scale model validation



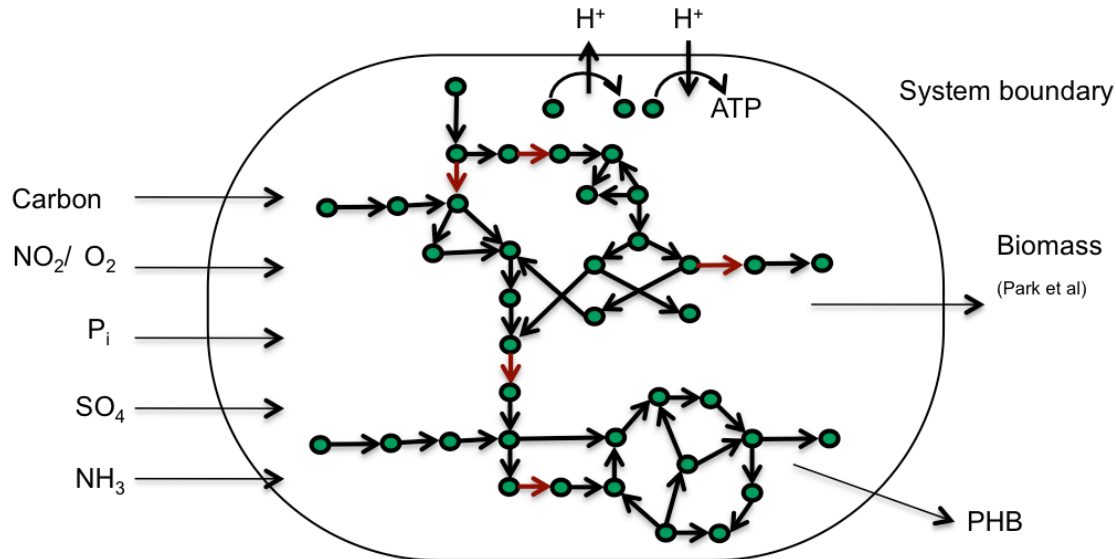
- Utilization of pathways:
 - ✓ Carbon dioxide fixed using **the Calvin cycle**
 - ✓ Fructose catabolised by **Entner-Doudroff** pathway

Genome scale model validation



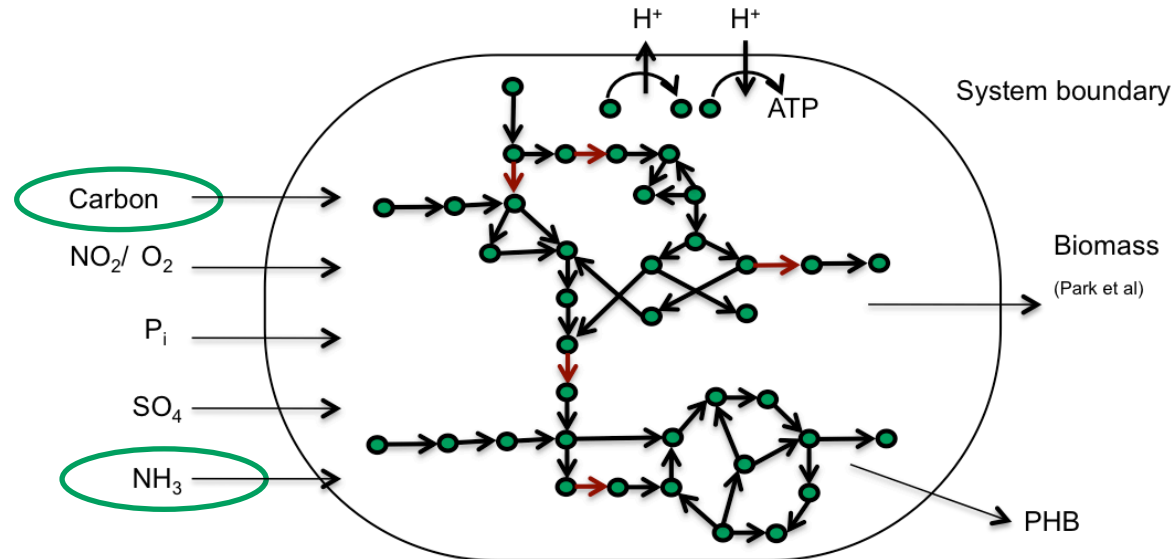
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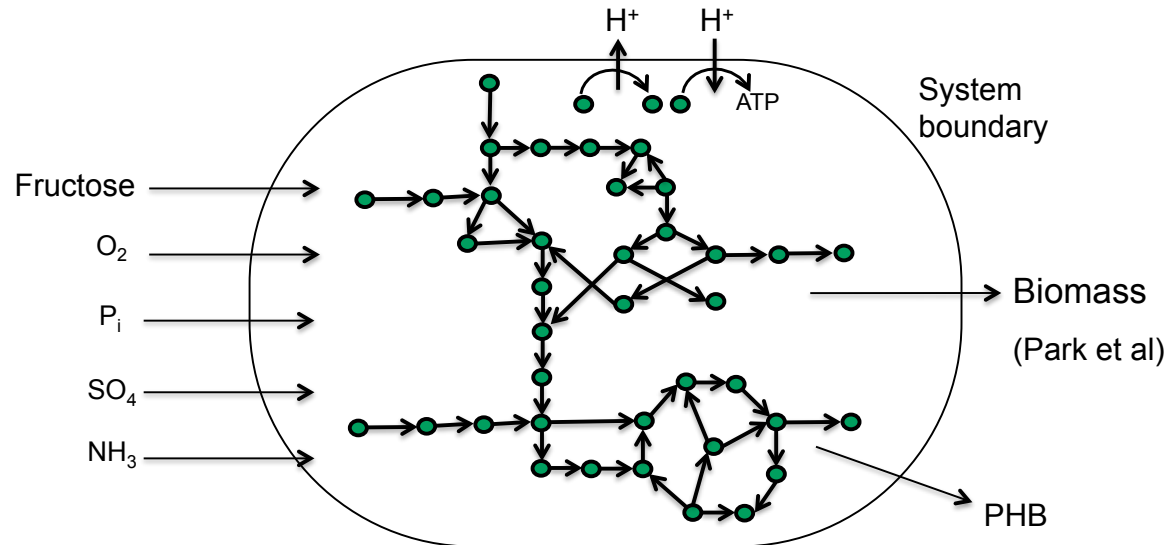
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- Growth on 90 substrates (e.g. Fructose, CO₂ + H₂, glycerol, butyrate, formate...)

Genome scale model validation



- Utilization of pathways:
 - ✓ Carbon dioxide fixed using **the Calvin cycle**
 - ✓ Fructose catabolised by **Entner-Doudroff** pathway
- Growth under **aerobic** and **anaerobic** conditions
- Growth on 90 substrates (e.g. Fructose, $\text{CO}_2 + \text{H}_2$, glycerol, butyrate, formate...)
- Carbon to nitrogen ratio (6.4 : 1.0)

Genome scale model validation

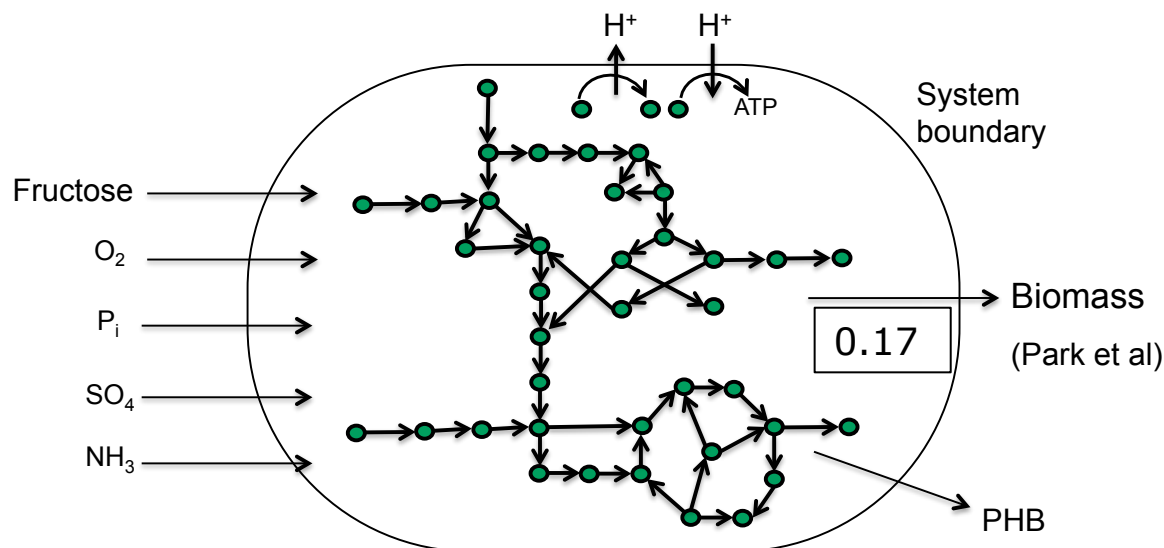


Substrate (mmol gDCW h)	Growth rate (1/h)
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Fructose (2.1 ± 0.3)	0.17 ± 0.03
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Glycerol (3.2 ± 0.1)	0.15 ± 0.04
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Genome scale model validation



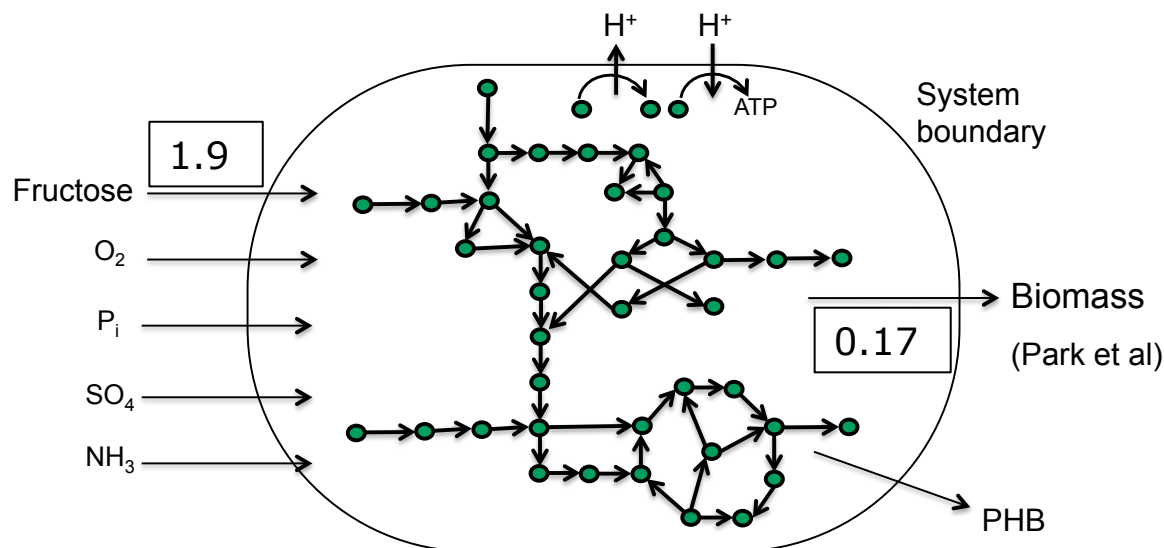
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minimize : $|\mathbf{v}|$

subject to :

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

Genome scale model validation



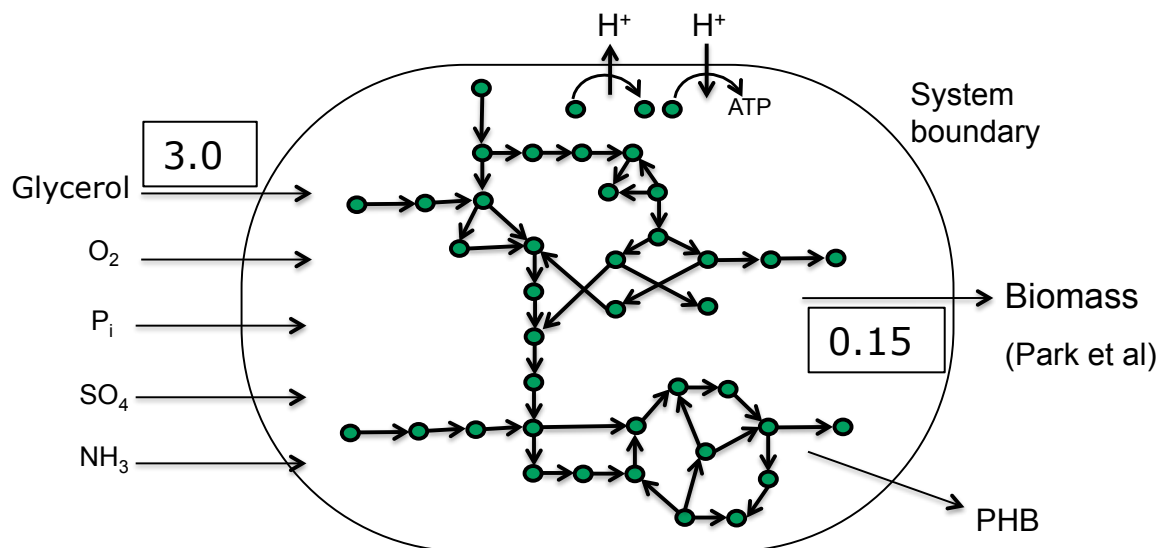
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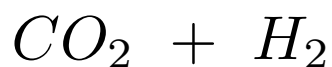
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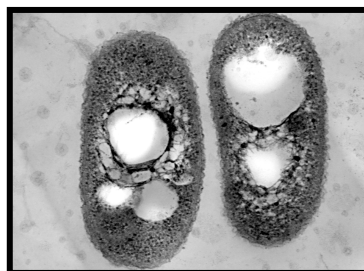
Assessing pathway yield and efficiency for platform chemical production

Waste into wealth using bacteria

Industrial waste

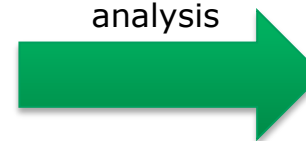


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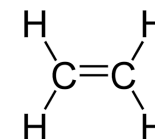


*Cupriavidus
necator*

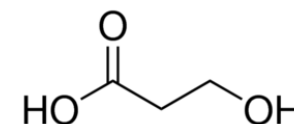
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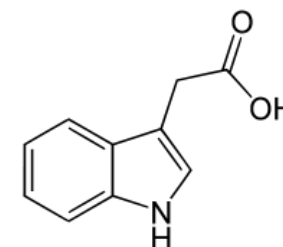
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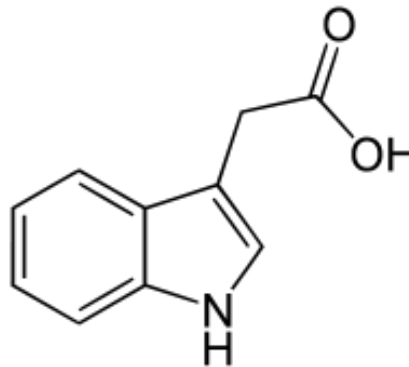
INDOLE-3-ACETATE



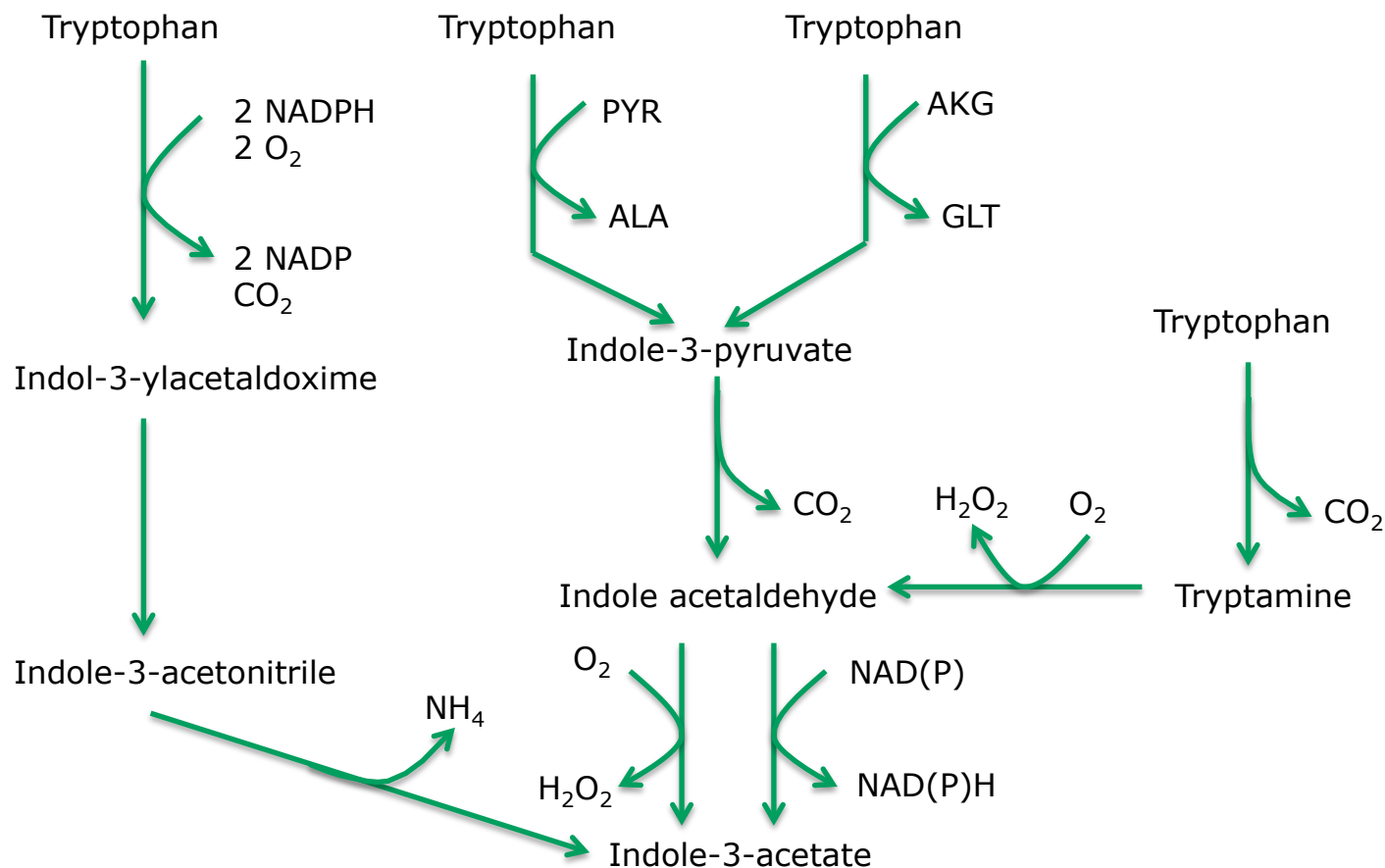
Indole-3-acetate (IAA)

Important **growth** and **development hormone** in multiple plant species, application is twofold:

- 1. Biofertilizer:** promotes **higher crop yields** and **protection against droughts** by increasing root length
- 2. Herbicide:** concentration dependent, overproduction of IAA **stunts plant growth**



Ranking IAA producing pathways



Maximum theoretical yields of IAA

maximise v_{IAA}

subject to :

$$\left\{ \begin{array}{l} Nv = 0 \\ 0 \leq v_{\text{CO}_2} \leq 5.0 \\ v_{\text{ATPase}} = J_{\text{ATPase}} \\ 0.05 \leq v_{\text{bio}} \leq \infty \end{array} \right.$$

Set objective function
to maximise flux to IAA
transporter

Maximum theoretical yields of IAA

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Steady state
assumption

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Constrain the CO_2 uptake rate to be less or equal to 5 $\text{mmol gDCW}^{-1}\text{h}^{-1}$

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Fix ATP
maintanance
costs

Maximum theoretical yields of IAA

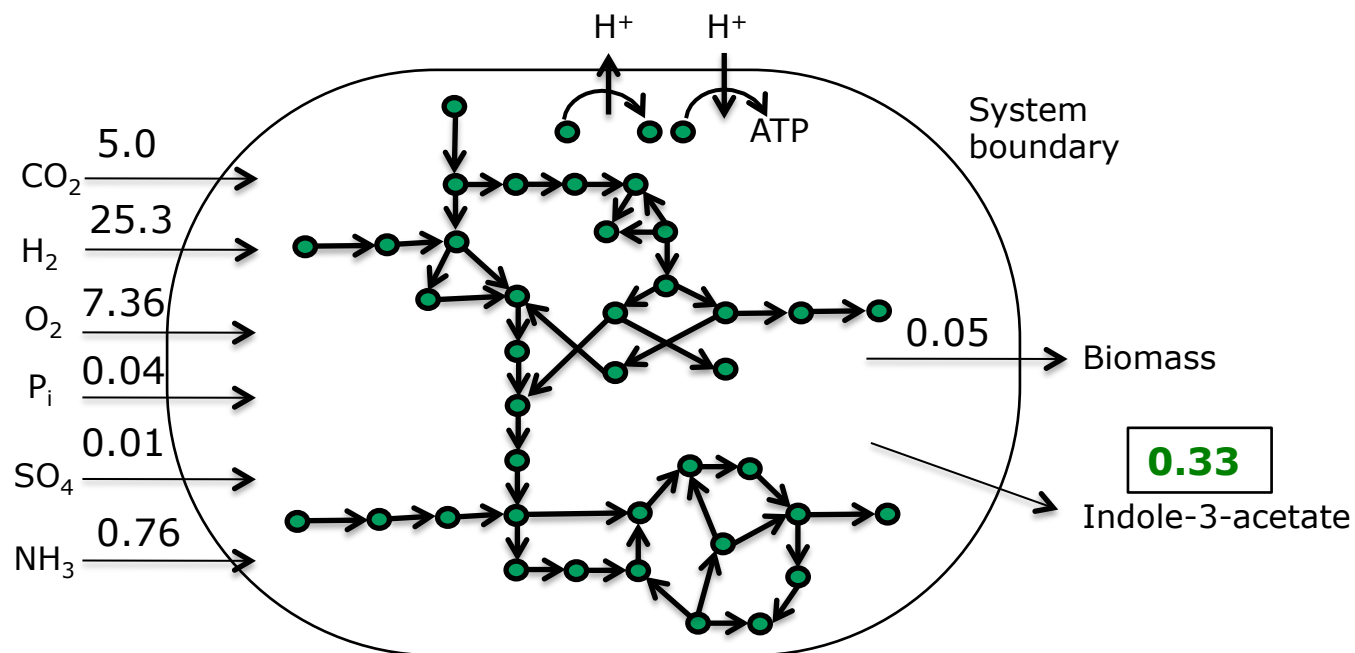
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Constrain biomass
to 0.05 h^{-1} or
higher.

Maximum theoretical yields of IAA



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

Minimum energy requirements

minimise v_{H_2}

subject to :

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← Set objective function
to minimise uptake flux
of hydrogen transporter

Minimum energy requirements

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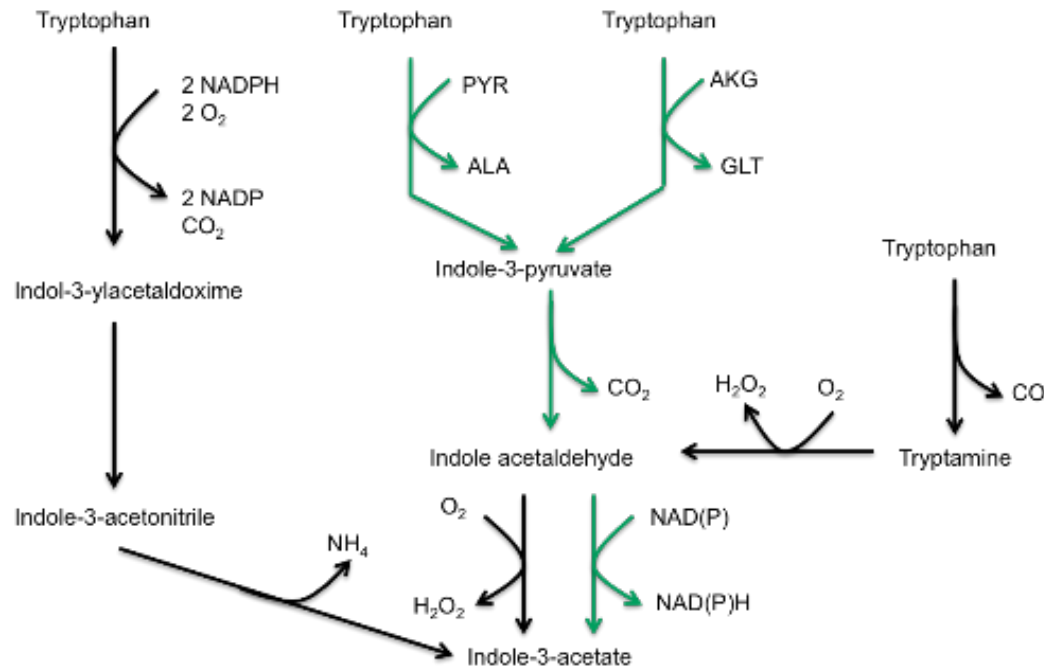
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Fix flux through IAA transporter to maximum theoretical value.

Minimum energy requirements

Pathway	1	2	3	4	5	6	7
Min. O ₂	7.76	7.76	8.14	7.73	7.36	7.36	7.56
H ₂ per IAA	78.72	78.72	81.09	78.55	76.55	76.55	77.55



Redirecting flux towards the target chemical

Identifying reaction/gene knock-outs

- **Elementary modes analysis.**

Identify all feasible solutions and then find **minimum** number of knock-outs that would **remove** all routes that **do not** produce the product. **Limited** to **small** networks.

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Loop through the reactions, **constraining** the flux of one (or a combination) of reaction(s) to **zero**. Combinations of knock-outs **increases exponentially**.

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Increase the flux of the **product** (e.g. from 0 to 5 mmol.gDCW⁻¹.h⁻¹) and identify the **reactions** whose flux **decreases**. Consider **knock-out combinations** of these changing reactions.

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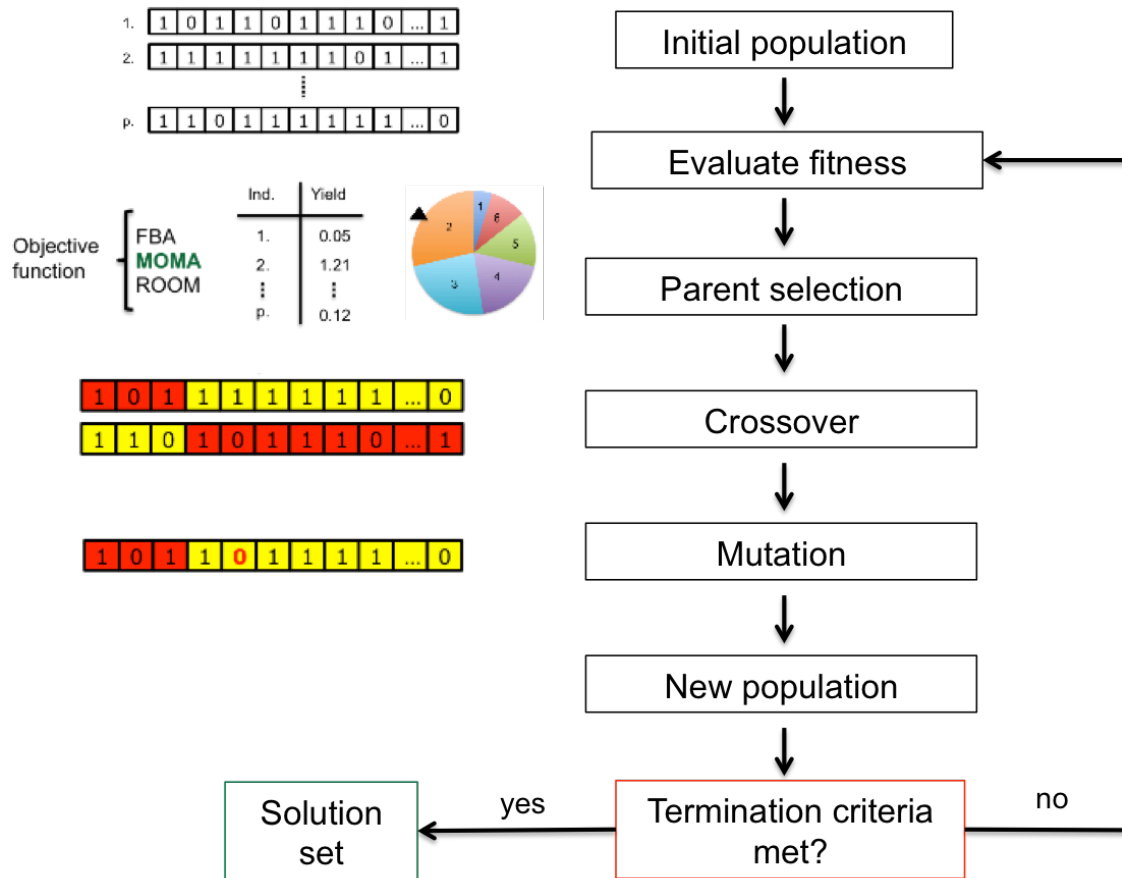
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- **Evolutionary algorithms (optGene).**

Bio-inspired algorithm for optimising the set of knock-outs in order to maximise chemical production. The solution is **not** guaranteed to be the best.

Identifying gene knockouts using OptGene



In Confidence

17

Identifying gene knockouts using OptGene

Objective function	No. of deletions	Suggested deletions	IAA yield
FBA/minimisation of sum of flux	4	Alanine-dehydrogenase, Alanine-aminotransferase, Arylformamidase B-alanine aminotransferase	16%
Minimisation of metabolic adjustments (MoMA)	6	Glutamate dehydrogenase, Arylformamidase, Phosphogluconolactonase, Methylglyoxal synthase, PEP synthase, Phosphoglycerate mutase	30%

Identifying gene knockouts using OptGene

- Requires **predefining** parameters (e.g. population size, mutation rate, number of knock-outs, objective function)
- May get 'stuck' in **local optima** and fail to find a solution, parameters may need adjusting or search space reducing
- No guarantee that the solution is **optimal**
- May not be a **unique** solution

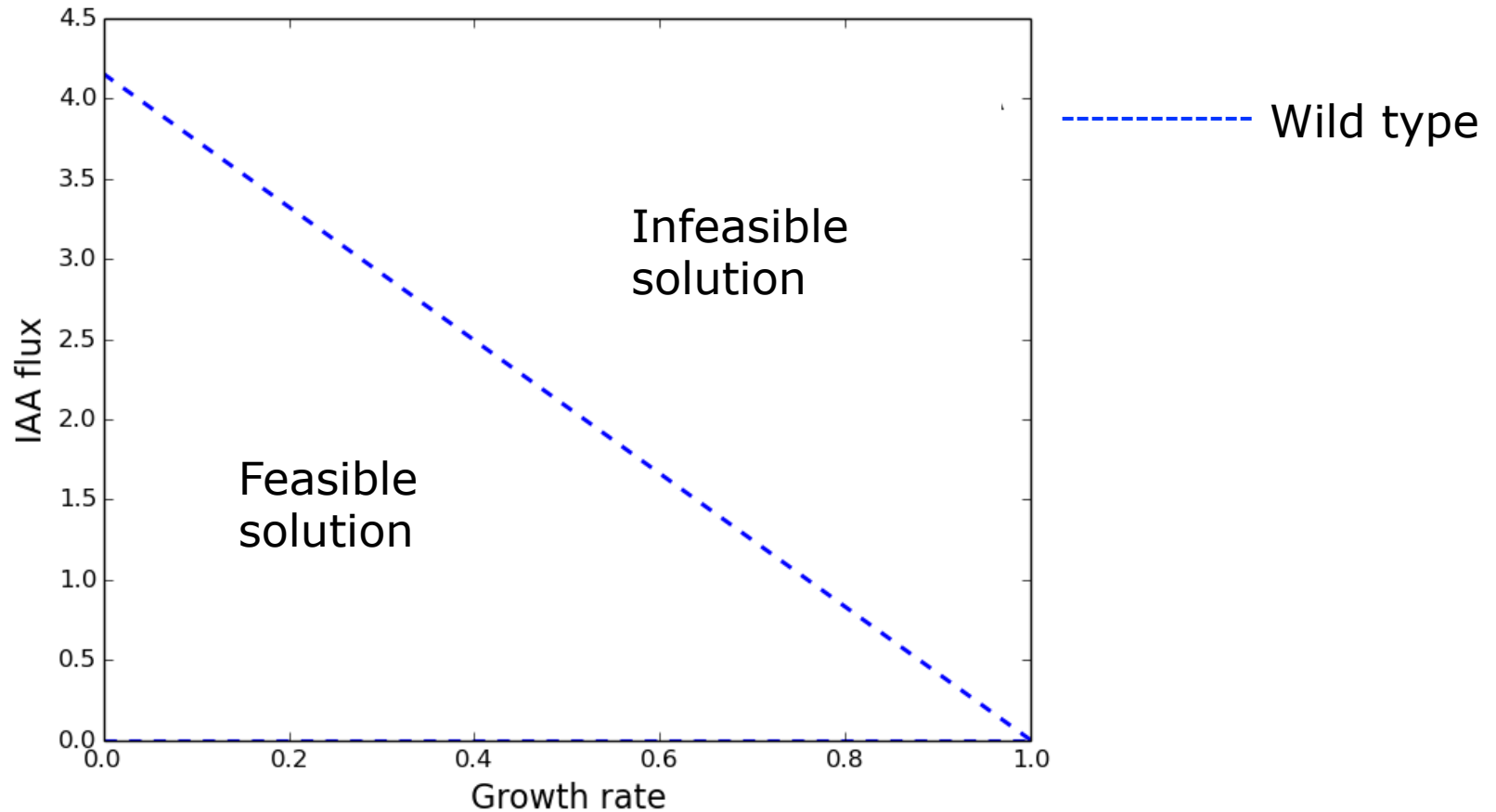
Assessing strategies using Flux Varied Analysis

maximise or minimise v_{IAA}

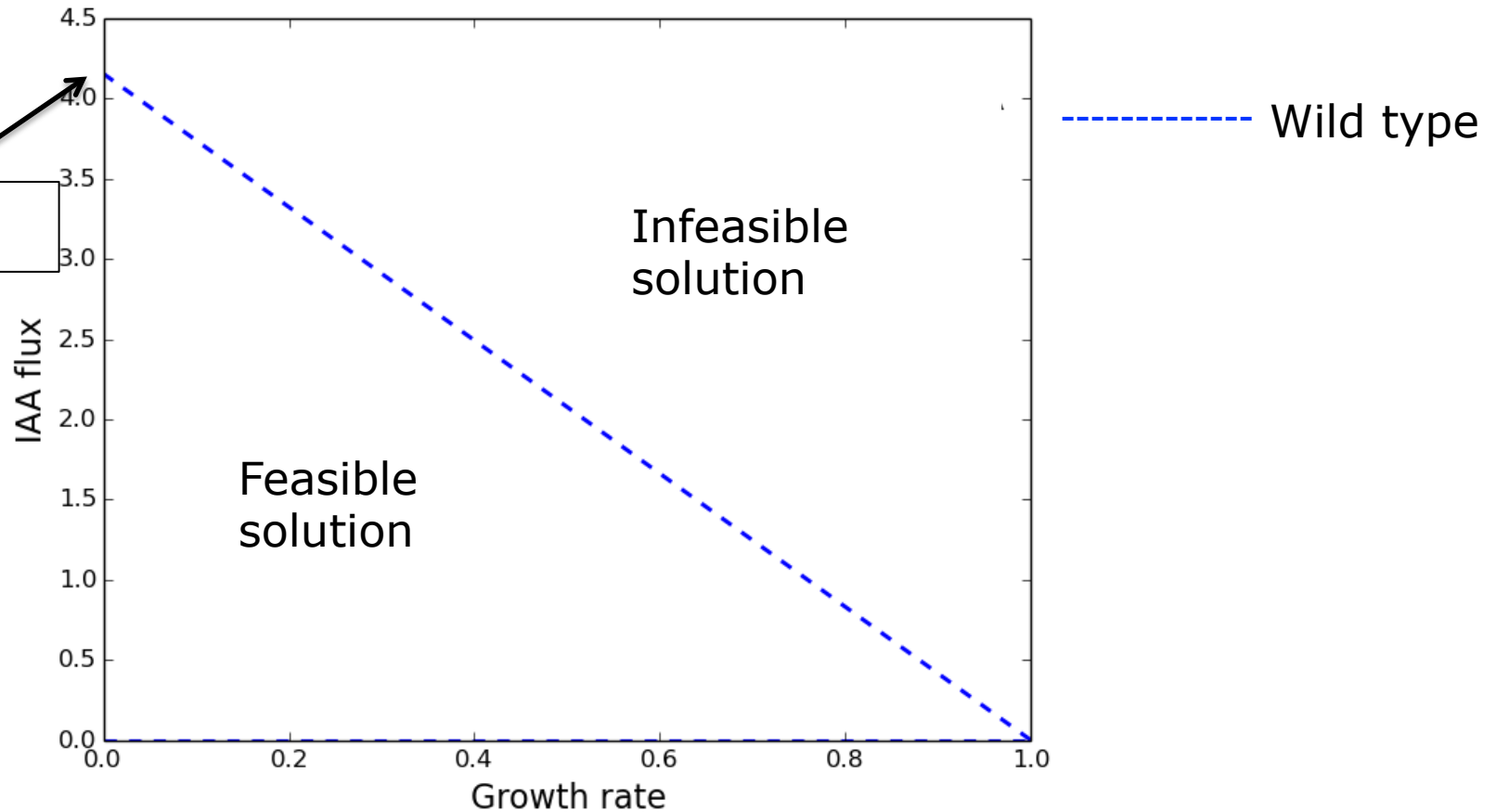
$$\text{subject to : } \begin{cases} N\mathbf{v} = 0 \\ v_j = t_j \\ v_{\text{ATPase}} = J_{\text{ATPase}} \\ v_{\text{ko}} = 0 \\ 0 \leq v_{\text{CO}_2} \leq 41.5 \end{cases} \leftarrow \begin{array}{l} \text{Constrain maximum} \\ \text{CO}_2 \text{ uptake} \end{array}$$

- LP resolved for increasing growth rates for both minimising and maximising IAA flux

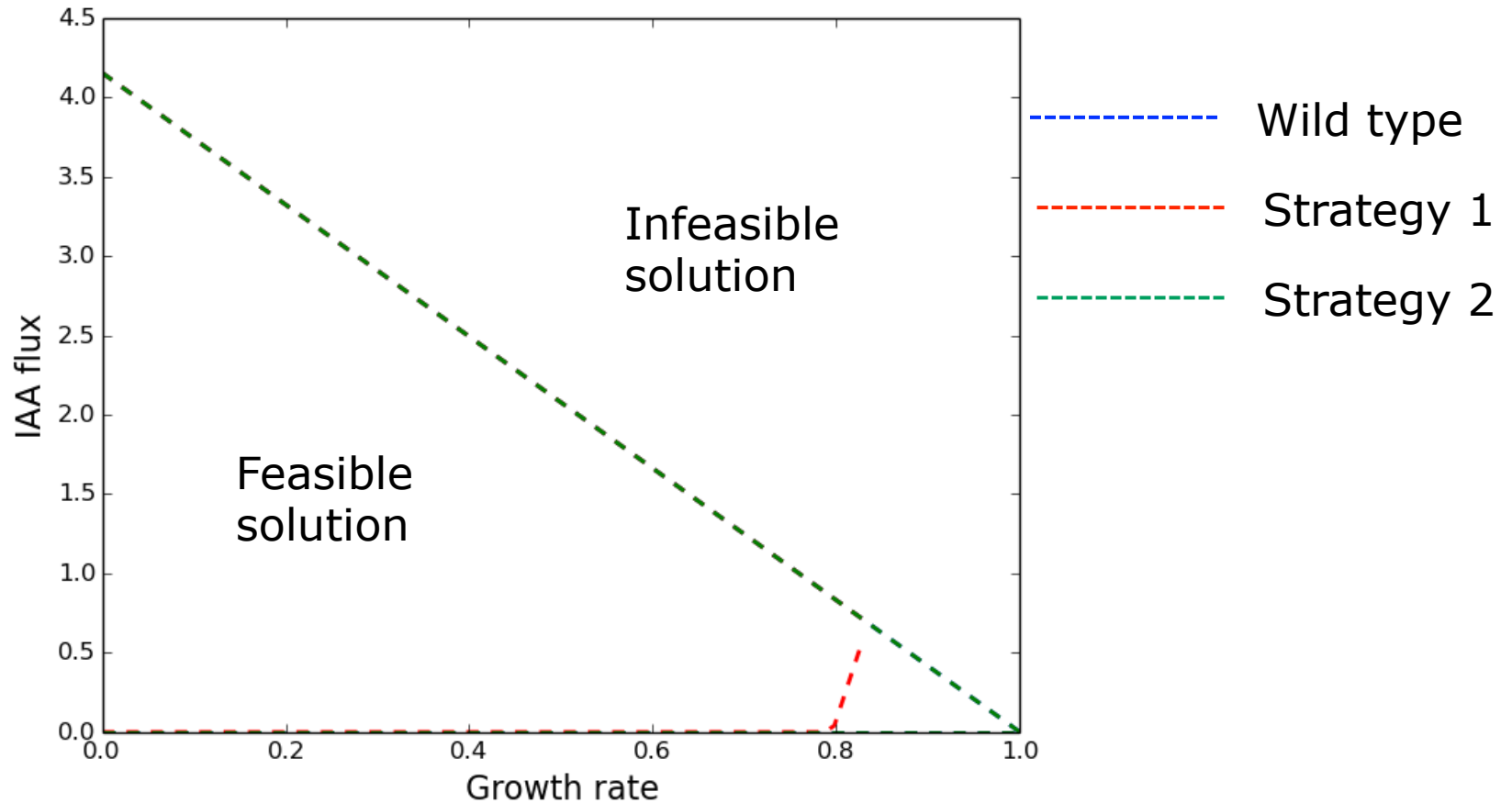
Assessing strategies using Flux Varied Analysis



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Assessing strategies using Flux Varied Analysis



Conclusions

- Assessed heterologous pathways using a GSM of *Cupriavidus necator* by calculating maximum theoretical yields and minimum energy requirements.
- Used an evolutionary algorithm (optGene) to identify potential knock-outs for redirecting flux to indole-3-acetate in *C. necator*.
- Flux varied analysis / production envelopes can then be used to assess the strategies likeliness of increasing flux to the target chemical.

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Jacque Minton
Alan Burbidge

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John King
Thomas Milat
Rupert Norman
Vanisha Patel
Jamie Twycross

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Kati Kovacs
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Freddi Walter