Applications of structural analysis of metabolic networks.

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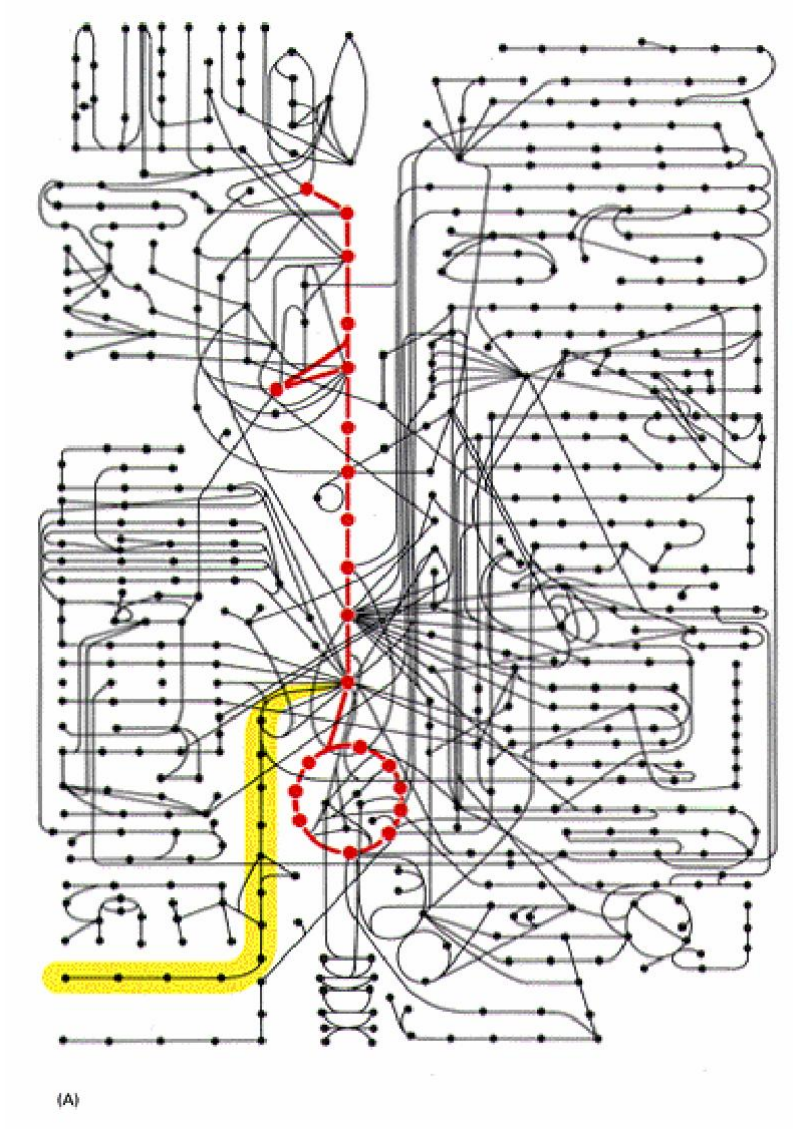
Outline

- **Theoretical approaches to the analysis of metabolic networks**
- Applications of elementary modes analysis:
  - Relating physiology to network structure
  - Identifying productive routes
  - Determining usage of modes
- Substructure in metabolic networks

The metabolic network



Pentose phosphate reactions

Gene	Reaction	*E. coli*
Pgi	G6P = F6P	EC4025
Pfk	F6P + ATP – ADP + FP2	EC3916
Fbp	FP2 - F6P + Pi	EC4232
Fba	FP2 = GAP + DHAP	EC2925
TpiA	GAP = DHAP	EC3919
Gap	GAP + Pi + NAD = NADH + BPG	EC1779
Pgk	BPG + ADP = ATP + P3G	EC2926
Gpm	P3G = P2G	EC0755
Eno	P2G = PEP	EC2779
Pyk	PEP + ADP – ATP + Pyr	EC1854
Zwf	G6P + NADP = GO6P + NADPH	EC1852
Pgl	GO6P – P6G	EC0766
Gnd	P6G + NADP = NADPH + CO2 + Ru5P	EC2029
Rpi	Ru5P = R5P	EC2914
Rpe	Ru5P = Xyl5P	EC3386
TktI	R5P + Xyl5P = GAP + Sed7P	EC2935
		EC2465
Tal	GAP + Sed7P = Ery4P + F6P	EC2464
TktII	Xyl5P + Ery4P = F6P + GAP	EC2935
		EC2465
Prs	R5P – R5Pex	EC4383

What pathways make up metabolism?

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Metabolic networks

If we can prepare a list of the reactions occurring in the metabolism of an organism, can we decide:

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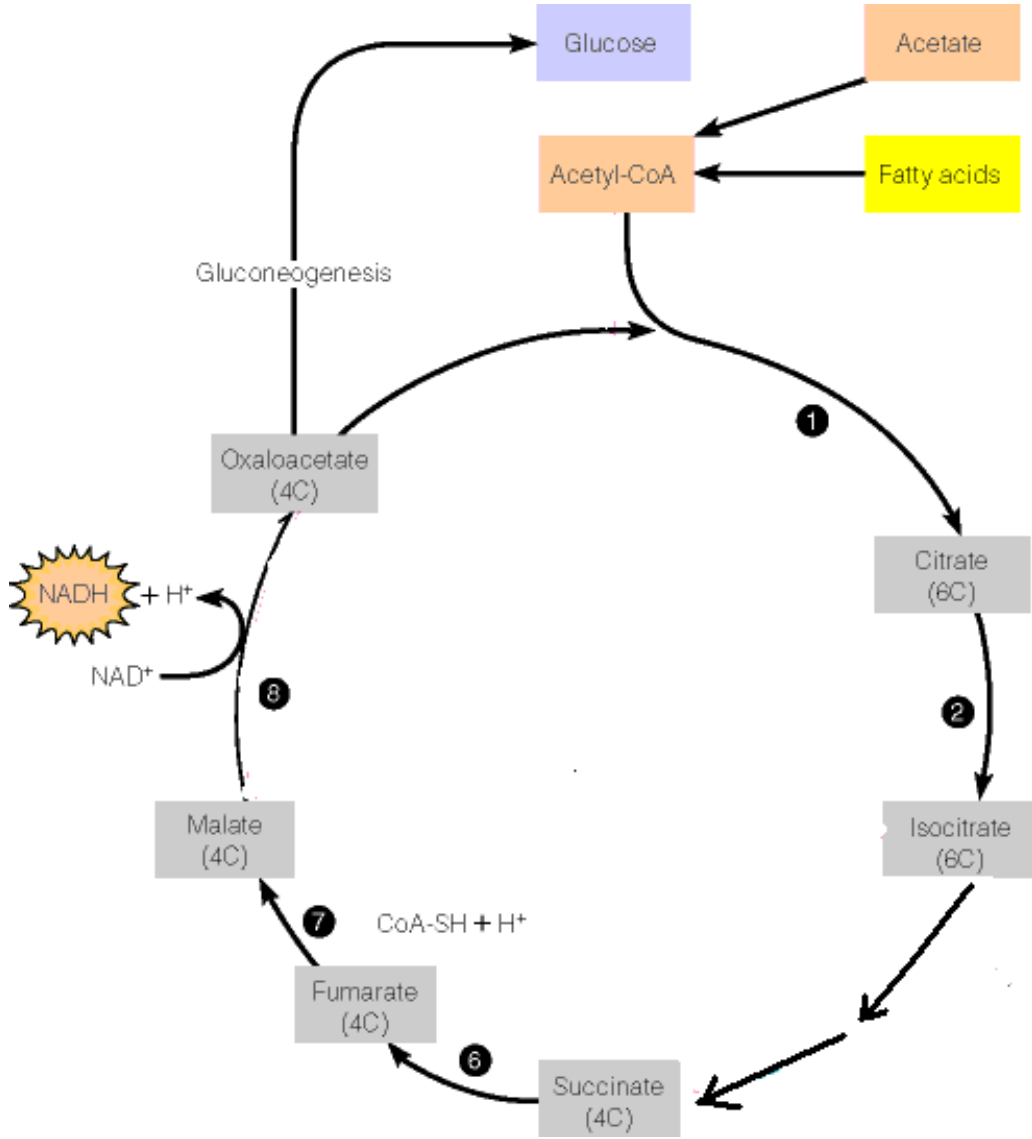
- what are the consequences of deleting an enzyme?
- whether genome annotations for an organism generate a connected and self-consistent metabolism?

Do graph algorithms have the answer?



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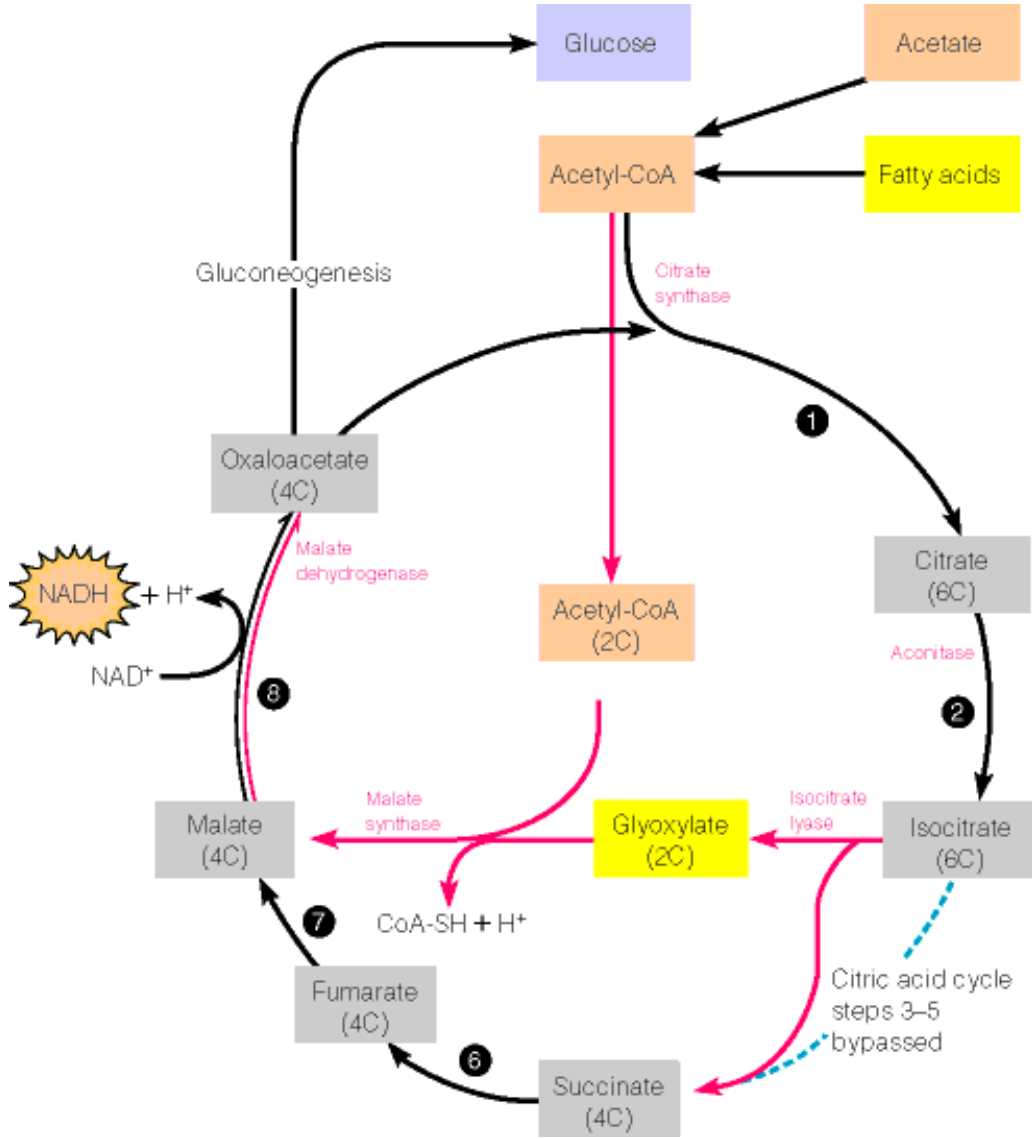
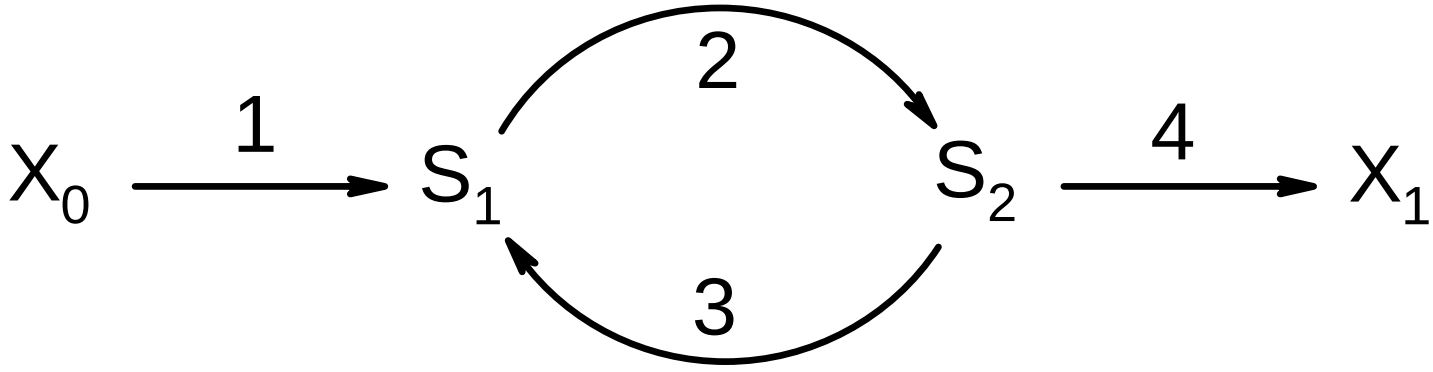


Representation

Consider a simple pathway, e.g.:



$$\begin{matrix} S_1 \\ S_2 \end{matrix} \begin{bmatrix} 1 & -1 & 1 & 0 \\ 0 & 1 & -1 & -1 \end{bmatrix}$$

r1: $X_0 \rightarrow S_1 \sim$
r2: $S_1 \rightarrow S_2 \sim$
r3: $S_2 \rightarrow S_1 \sim$
r4: $S_2 \rightarrow X_1 \sim$

Separating structure and kinetics

The rate at which the substrate concentrations are changing is given by $\mathbf{N}.\mathbf{v}$, where $\mathbf{N}$ is the stoichiometry matrix, and $\mathbf{v}$ are the enzyme kinetic functions. So for our substrate cycle pathway:

$$\begin{bmatrix} \frac{dS_1}{dt} \\ \frac{dS_2}{dt} \end{bmatrix} = \begin{bmatrix} 1 & -1 & 1 & 0 \\ 0 & 1 & -1 & -1 \end{bmatrix} . \begin{bmatrix} v_1 \\ v_2 \\ v_3 \\ v_4 \end{bmatrix}$$

where each v_i is the rate function for enzyme i, depending on the metabolites, V_{m}, K_{m} etc.

Steady state solutions

Any metabolic pathway at steady state satisfies the relationship $\mathbf{N}.\mathbf{v} = \mathbf{0}$, where $\mathbf{N}$ is the stoichiometry matrix, exemplified by the substrate cycle pathway:

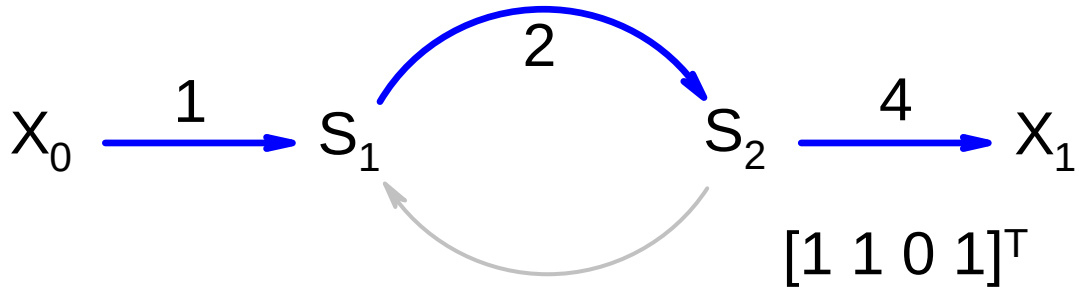
$$\begin{array}{c} S_1 \\ S_2 \end{array} \begin{bmatrix} 1 & -1 & 1 & 0 \\ 0 & 1 & -1 & -1 \end{bmatrix} . \begin{bmatrix} v_1 \\ v_2 \\ v_3 \\ v_4 \end{bmatrix} = \begin{bmatrix} 0 \\ 0 \end{bmatrix}$$

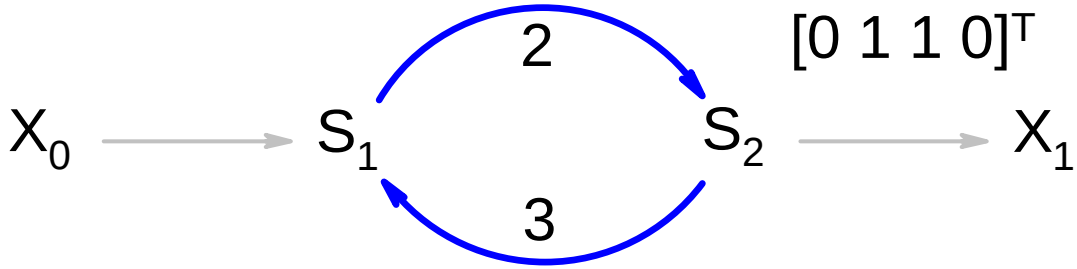
Steady state solutions – 2

Any observed set of velocities at steady state will be a linear combination of a set of vectors $\mathbf{K}$ referred to as the null space of the stoichiometry matrix. In this case,

$$\mathbf{K} = \begin{bmatrix} 1 & 0 \\ 1 & 1 \\ 0 & 1 \\ 1 & 0 \end{bmatrix}$$

Null space vectors as pathways





$[1 \;\; 1 \;\; 0 \;\; 1]^T$ and $[0 \;\; 1 \;\; 1 \;\; 0]^T$

Steady state solutions – 3

Any feasible set of velocities at steady state is a linear combination of these null space vectors, e.g.:

$$\mathbf{K} = \begin{bmatrix} 1 & 0 \\ 1 & 1 \\ 0 & 1 \\ 1 & 0 \end{bmatrix}$$

and:

$$\begin{bmatrix} 1 & 0 \\ 1 & 1 \\ 0 & 1 \\ 1 & 0 \end{bmatrix} \cdot \begin{bmatrix} a & b \end{bmatrix} = \begin{bmatrix} a \\ a+b \\ b \\ a \end{bmatrix} = \begin{bmatrix} v_1 \\ v_2 \\ v_3 \\ v_4 \end{bmatrix}$$

Problems with the null space

Shortcomings of the set of basis vectors as metabolic routes:

- Is not a unique solution
- May not respect thermodynamic direction
- Not necessarily ’simple’
- Can mislead about the impact of enzyme deletion

The linear programming approach

The substrate cycle pathway can also be expressed in terms of the full stoichiometry matrix $\mathbf{N}$:

$$\begin{array}{c} X_0 \\ S_1 \\ S_2 \\ X_1 \end{array} \begin{bmatrix} -1 & 0 & 0 & 0 \\ 1 & -1 & 1 & 0 \\ 0 & 1 & -1 & -1 \\ 0 & 0 & 0 & 1 \end{bmatrix} \cdot \begin{bmatrix} v_1 \\ v_2 \\ v_3 \\ v_4 \end{bmatrix} = \begin{bmatrix} -x \\ 0 \\ 0 \\ \geq 0 \end{bmatrix}$$

Linear programming gives a single route satisfying a particular set of constraints and an optimization function. It does not tell of other similar routes.

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An elementary mode is a minimal set of enzymes that can operate at steady state with all irreversible reactions working in the thermodynamically favoured direction, and enzymes weighted by the *relative* flux they carry.

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Hence the set of elementary modes of a reaction network is unique.

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Crassulacean acid metabolism

This is a specialized form of photosynthesis adopted by some plants that live in arid areas, and that collect CO_2 by night and close their stomata by day to save water.

- Considering phase 3 of CAM metabolism - daylight metabolism of stored malate with no net CO_2 uptake.
- CAM plants can be divided into 2 groups according to whether malic enzyme or PEP carboxykinase is the main enzyme of malate utilization.
- Christopher & Holtum (1996) proposed a further division into producers of chloroplastic starch and producers of extrachloroplastic sucrose or hexose, giving 4 categories.

Elementary modes analysis of CAM

- There are 6 elementary modes, not 4; 3 for malic enzyme, 3 for PEPCK plants.
- Production of hexose and starch together is a distinct route from production of either starch or hexose, differing in transport between chloroplast and cytosol.
- *Aloe vera* is an example, given by the original authors, of a PEPCK plant forming both starch and hexose.
- The missing class: a malic enzyme plant forming both starch and hexose - *Mesembryanthemum crystallinum*. (Thanks to Dr Annie Borland, Newcastle, for finding this for me.)

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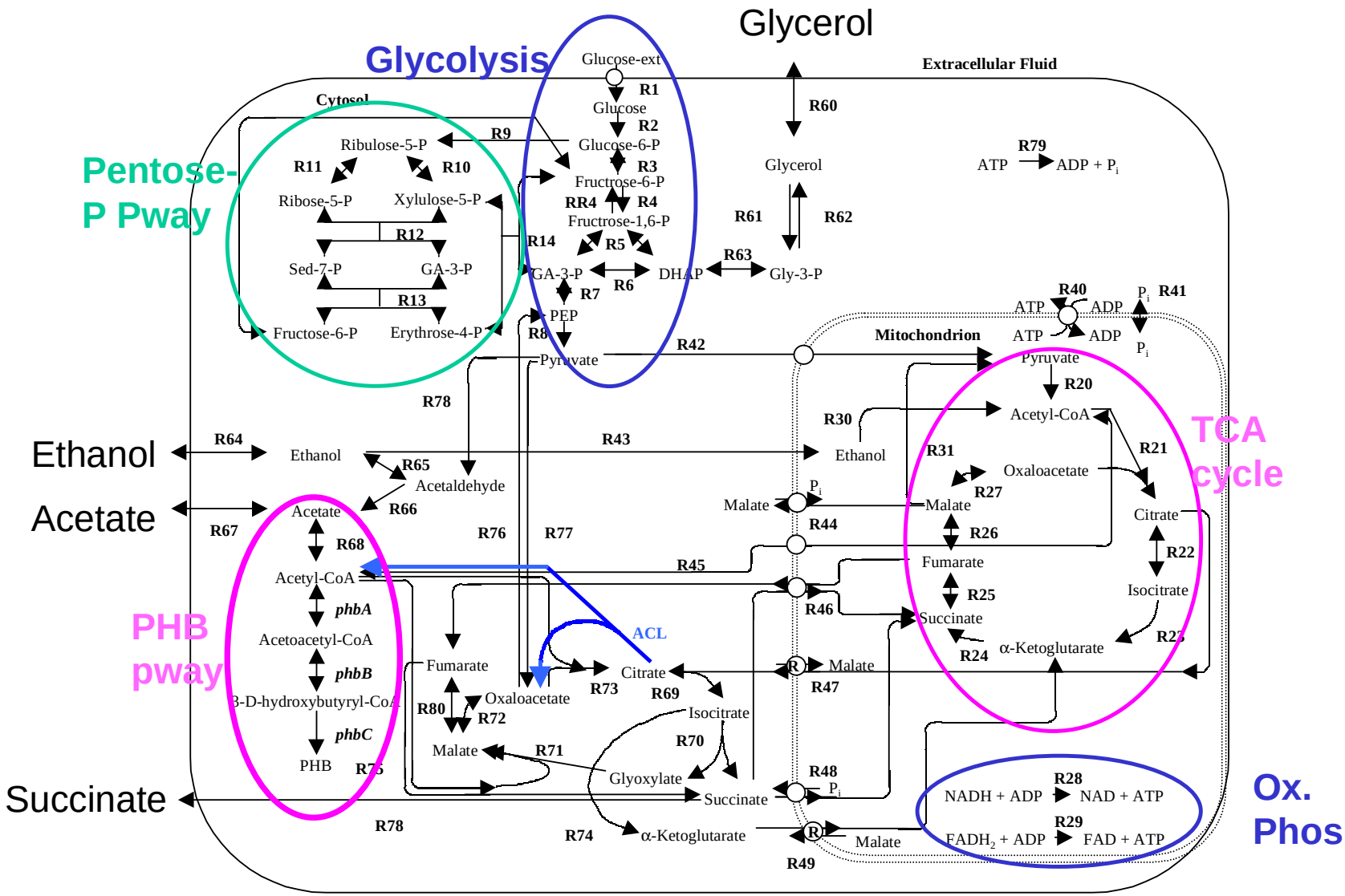
Polyhydroxybutyrate synthesis in yeast



Optimal yields of PHB synthesis

Wild-type yeast + PHB pathway

1. 2 Acetate + EtOH $\rightarrow$ PHB + 2 CO_2 0.67
2. 65 Ac. + 31 EtOH $\rightarrow$ 30 PHB + 72 CO_2 0.63

(Number following each mode is the fractional carbon conversion.)

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Wild-type yeast + ATP–citrate lyase + PHB pathway

3. 12 EtOH $\rightarrow$ 5 PHB + 4 CO_2 0.83
4. 77 EtOH + 31 Glycerol $\rightarrow$
   48 PHB + 4 Ac. + 47 CO_2 0.78

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Relative flux in elementary modes

The steady state requirement is:

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So the actual velocities, $\mathbf{v}$ are a weighted combination of the elementary modes:

$$\mathbf{v} = \mathbf{E}.\mathbf{w}$$

where $\mathbf{w}$ is a vector of weighting factors.

Solutions for the weighting vector $\mathbf{w}$

Although there is not a unique solution for $\mathbf{w}$ because $\mathbf{E}$ is generally non–invertible and the system under–determined:

$$\hat{\mathbf{w}} = \mathbf{E}^{\#}\mathbf{v}$$

where $\mathbf{E}^{\#}$ is the generalised Penrose inverse of $\mathbf{E}$. Even if we have not measured all the fluxes in $\mathbf{v}$, we can partition observed $\mathbf{v}_o$ and non–observed $\mathbf{v}_x$ fluxes:

$$\mathbf{v} = \left[\mathbf{v}_o, \mathbf{v}_x\right]^T, \text{ and } \mathbf{E} = \begin{bmatrix} \mathbf{E}_o \\ \mathbf{E}_x \end{bmatrix}.$$

Then:

$$\hat{\mathbf{w}} = \mathbf{E}_o^{\#}\mathbf{v}_o$$

Properties of the solution for $\hat{\mathbf{w}}$

Why select $\hat{\mathbf{w}}$ from the many feasible values of $\mathbf{w}$?

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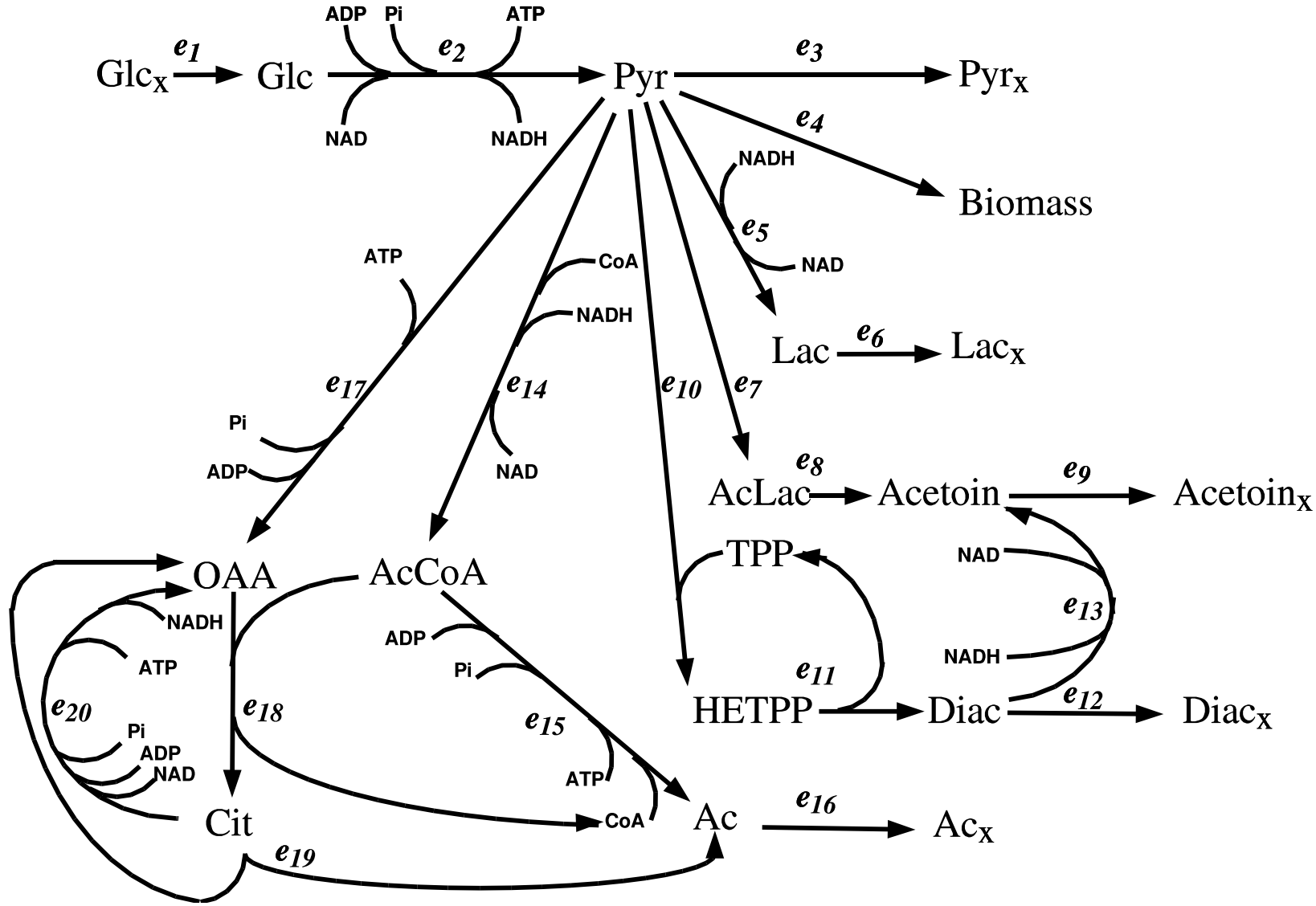
- It assigns flux as evenly as possible over available modes (the ‘democratic’ option).
- When $\mathbf{v}_o$ changes smoothly, so does $\hat{\mathbf{w}}$. (Not the case for other extreme solutions, e.g. the ‘autocratic’ option.)

Flux analysis of lactic acid metabolism



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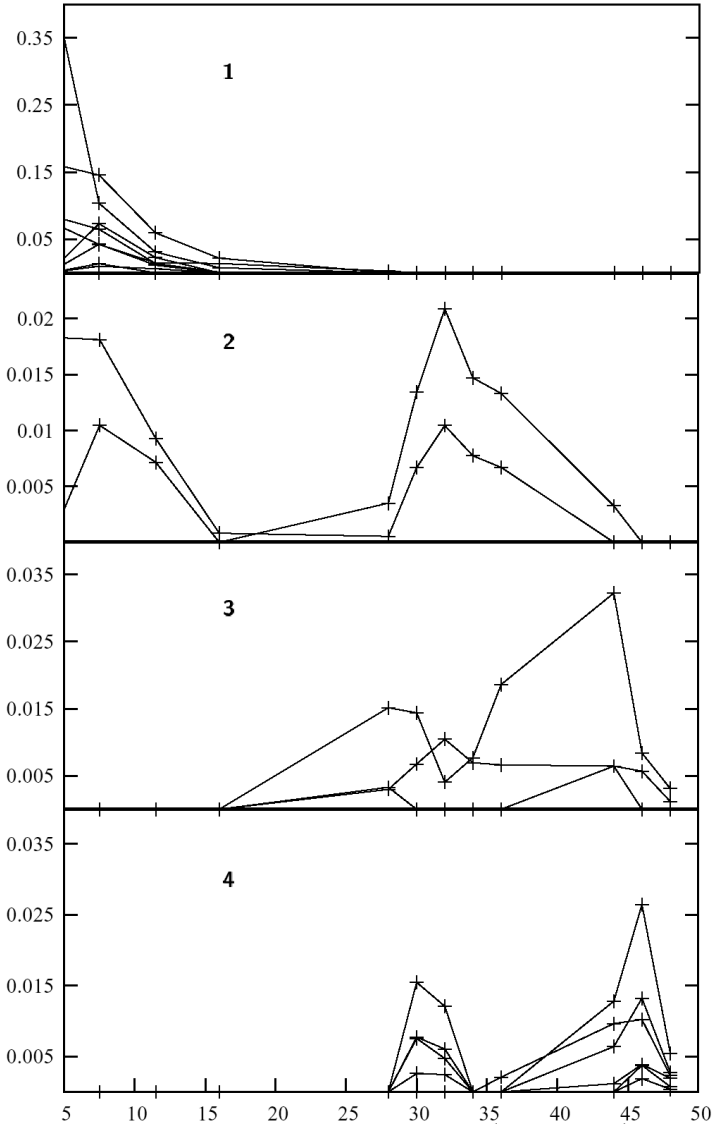
- The time–dependence of these fluxes defined 4 groups.

Mode assignment



Stoichiometry	Peak flux mol.hr^{-1}.(Mol. Org.)$^{-1}$
Group 1	
Glc $\longrightarrow$ 2 Lac	0.45
1/2 O_2 + Glc $\longrightarrow$ 2 Biomass	0.16
1/2 O_2 + Glc $\longrightarrow$ 2 CO_2 + Acetoin	0.086
O_2 + Glc $\longrightarrow$ 2 Ac + 2 CO_2	0.077
1/2 O_2 + Glc $\longrightarrow$ 2 Pyr	0.073
Pyr $\longrightarrow$ 5/6 Lac + 1/2 CO_2	0.042
3/4 O_2 + Glc $\longrightarrow$ Diac + 2 CO_2	0.014
3/4 O_2 + Glc $\longrightarrow$ Cit	0.01
3 O_2 + Glc $\longrightarrow$ 6 CO_2	0.01
Glc + 6 Pyr $\longrightarrow$ 4 Lac + 2 Cit	0.0003

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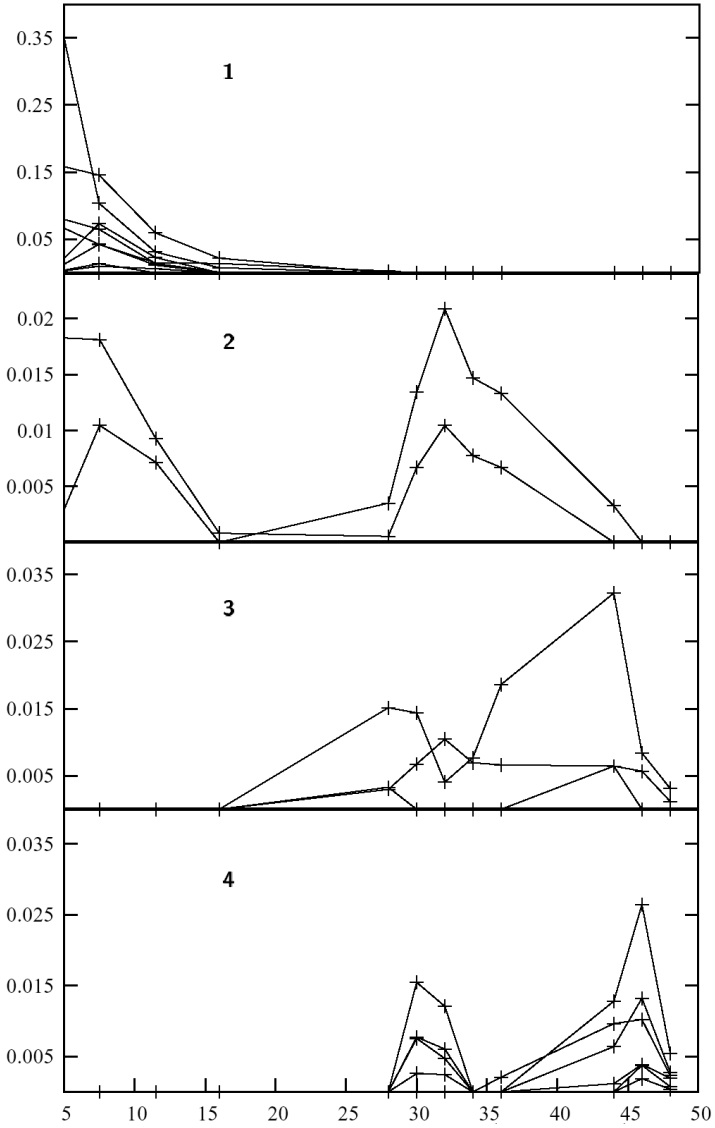
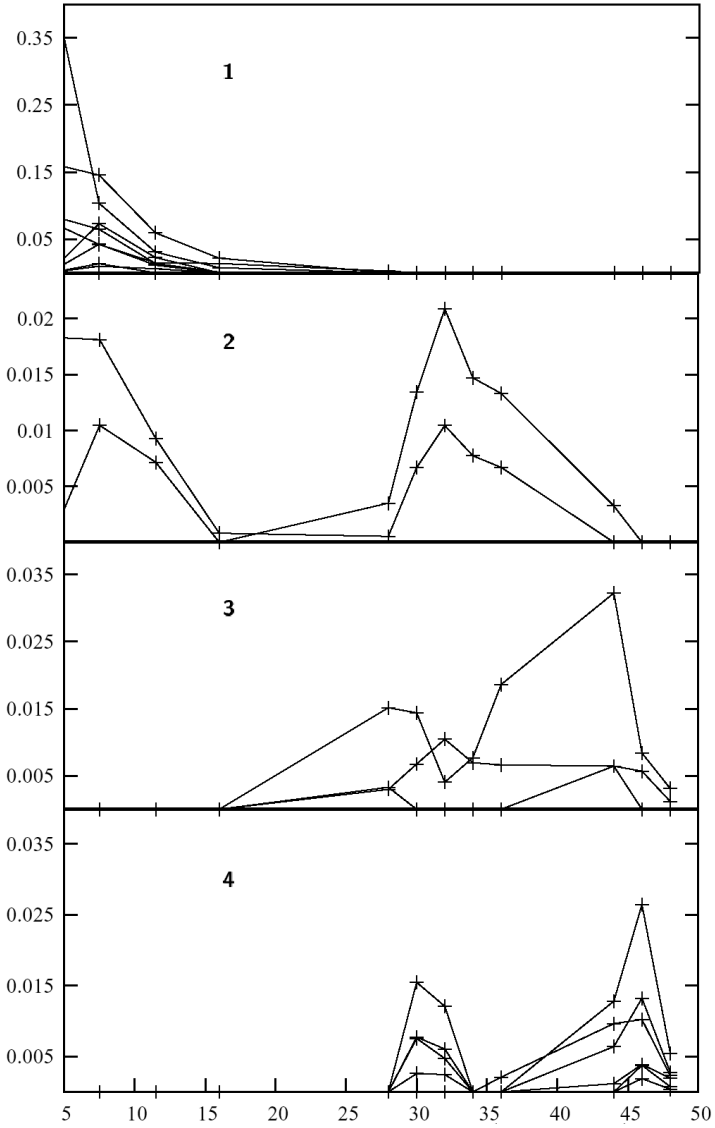


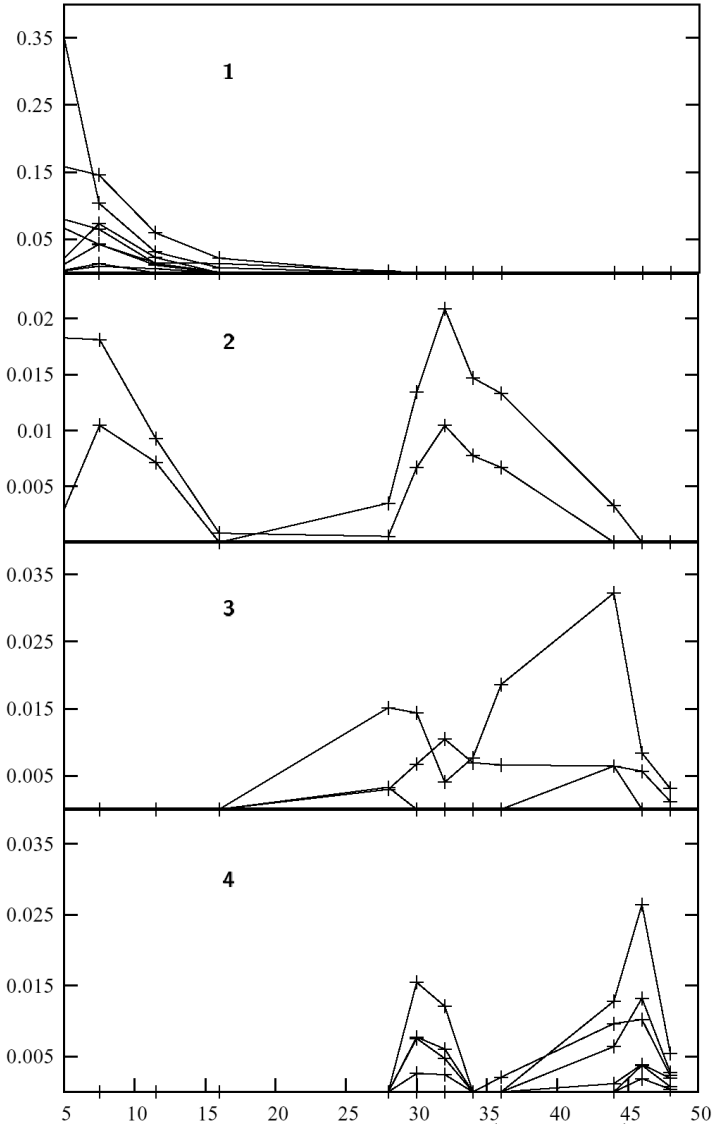
Stoichiometry	Peak flux $mol.hr^{-1}.(Mol.\ Org.)^{-1}$
Group 2	
Lac 1/4 + O_2 $\longrightarrow$ Biomass	0.021
Pyr $\longrightarrow$ Biomass	0.018

Mode assignment



Stoichiometry	Peak flux $mol.hr^{-1}.(Mol.\ Org.)^{-1}$
Group 3	
Lac 1/2 + O_2 $\longrightarrow$ Ac + CO_2	0.032
Lac 1/4 O_2 $\longrightarrow$ Pyr	0.01
1/4 O_2 + Pyr $\longrightarrow$ Ac + CO_2	0.006

Mode assignment



Stoichiometry			Peak flux $mol.hr^{-1}.(Mol.\ Org.)^{-1}$
Group 4			
Lac + 3/2 O_2	$\longrightarrow$	3 CO_2	0.026
Lac + 3/4 O_2	$\longrightarrow$	1/3 Cit + CO_2	0.013
Lac + 5/12 O_2	$\longrightarrow$	1/3 Cit + 1/3 Ac + 1/3 CO_2	0.010
Lac + 1/4 O_2	$\longrightarrow$	$CO_2$1/2 Acetoin	0.0039
5/4 O_2 + Pyr	$\longrightarrow$	3 CO_2	0.0038
1/2 O_2 + Pyr	$\longrightarrow$	1/3 Cit + CO_2	0.0019

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- Prediction of possible co–regulation/co–expression patterns..

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- **Minnesota:** Friedrich Srienc, Ross Carlson
- **Mumbai:** K V Venkatesh

Further details

References:

- S Schuster, T Dandekar & D A Fell, Trends in Biotechnology, 17, 53-60 (1999).
- S Schuster, D A Fell & T Dandekar, Nature Biotechnol. 18, 326-332 (2000)

Algorithm:

- http: //mudshark.brookes.ac.uk/algorithm.pdf

Programs:

- ScrumPy: http://mudshark.brookes.ac.uk/ScrumPy/
- Metatool: http://www.bioinf.mdc-berlin.de/projects/metabolic/metatool/