Regulatory Flux Balance Analysis (rFBA)

Metabolische Netzwerke SS 14

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Iterated flux balance analysis

Varma/Palsson 1994

Flux balance model

$$S \cdot v = b$$

(b net metabolic uptake)

Objective

$$\text{minimize } Z = -v_{growth}$$

$$\sum_{\text{all}M} d_M \cdot M \xrightarrow{v_{growth}} \text{biomass}$$

Divide experimental time into small time steps Δt.

Specify initial values for *external* concentrations.

Use flux balance model to predict concentrations for the next step (⇨ dynamic profiles).

Iterative algorithm

1. Determine substrate concentration S_c from previous substrate concentration S_{co} and additional supply:

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

2. Scale substrate concentration

$$\text{Substr\_avail} = \frac{S_c}{X \cdot \Delta t}$$

(X is the cell density)

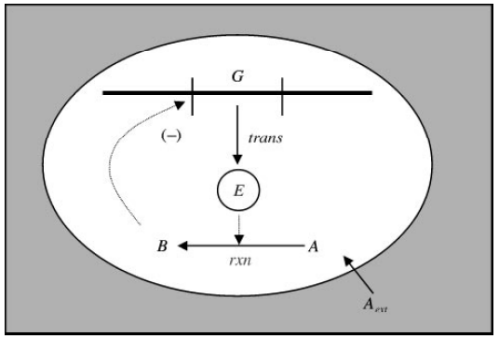
3. Use FBA to determine actual substrate uptake rate S_u, growth rate μ, and potential by-product secretion.

4. Compute new concentrations

$$\frac{dX}{dt} = \mu X \quad \Rightarrow \quad X = X_0 \cdot e^{\mu \Delta t}$$

$$\frac{\partial S_c}{\partial t} = -S_u \cdot X \quad \Rightarrow \quad S_c = S_{co} + \frac{S_u}{\mu} X_0 (1 - e^{\mu \Delta t})$$

Regulatory constraints



trans = IF (G) AND NOT (B)

rxn = IF (A) AND (E)

Gene *G* is transcribed by a process *trans* to produce an enzyme *E*.

This enzyme then catalyses a reaction *rxn* which converts substrate *A* into product *B*.

Product *B* then represses transcription of *G*, leading to depletion of *E*.

Regulatory flux balance analysis (rFBA)

Covert/Schilling/Palsson 2001

Refinement of iterative FBA

Divide experimental time into small time steps Δ t.

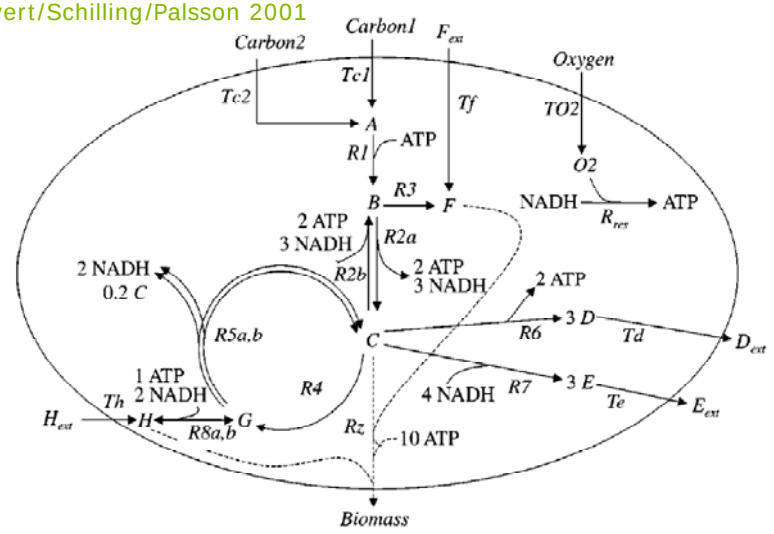
Reactions may happen in a given time interval $[t_1,t_2]$, if corresponding regulatory constraints are satisfied.

If a regulatory constraint for reaktion k does not hold in $[t_1,t_2]$, we impose the temporary constraint

$v_k(t) = 0$ when $t \in [t_1,t_2]$.

Simplified core carbon metabolism

Covert/Schilling/Palsson 2001



Mathematical model I

Metabolic reactions

$-1\ A - 1$ ATP $+ 1\ B$	*R1*
$-1\ B + 2$ ATP $+ 2$ NADH $+ 1\ C$	*R2a*
$-1\ C - 2$ ATP $- 2$ NADH $+ 1\ B$	*R2b*
$-1\ B + 1\ F$	*R3*
$-1\ C + 1\ G$	*R4*
$-1\ G + 0.8\ C + 2$ NADH	*R5a*
$-1\ G + 0.8\ C + 2$ NADH	*R5b*
$-1\ C + 2$ ATP $+ 3\ D$	*R6*
$-1\ C - 4$ NADH $+ 3\ E$	*R7*
$-1\ G - 1$ ATP $- 2$ NADH $+ 1\ H$	*R8a*
$+1\ G + 1$ ATP $+ 2$ NADH $- 1\ H$	*R8b*
-1 NADH $- 1\ O2 + 1$ ATP	*Rres*

Transport processes

-1 *Carbon1* $+ 1\ A$	*Tc1*
-1 *Carbon2* $+ 1\ A$	*Tc2*
$-1\ F_{ext} + 1\ F$	*Tf*
$-1\ D + 1\ D_{ext}$	*Td*
$-1\ E + 1\ E_{ext}$	*Te*
$-1\ H_{ext} + 1\ H$	*Th*
-1 *Oxygen* $+ 1$ *O2*	*To2*

Maintenance and growth processes

$-1\ C - 1\ F - 1\ H - 10$ ATP $+ 1$ *Biomass*	*Growth*

Preferential carbon source uptake

Assume *Carbon1* to be the preferred carbon source.

Presence of extracellular *Carbon1* activates a regulatory protein which inhibits the transcription of the gene which encodes a protein for transport of *Carbon2* into the cell, via a transport process *Tc2*.

RPc1 is the regulatory protein which senses extracellular *Carbon1*,

tTc2 is the occurrence of a transcription event (which will eventually result in the protein enabling transport process *Tc2* and the relaxation of one regulatory constraint, $v_{Tc2} = 0$).

RPc1 = *IF* (*Carbon1*)
tTc2 = *IF NOT* (*RPc1*)

Anaerobic growth

The transcription of many enzymes is regulated according to whether or not oxygen is available to the cell

Here, the presence of *Oxygen* will inactivate regulatory protein *RPO2*, which inhibits transcription of the genes for *Rres* and *R5a* but induces transcription of the gene for *R5b*.

R5a and *R5b* are reactions catalyzed by isozymes.

$$RPO2 = IF\ NOT\ (Oxygen)$$
$$tRres = IF\ NOT\ (RPO2)$$
$$tR5a = IF\ NOT\ (RPO2)$$
$$tR5b = IF\ (RPO2).$$

Amino acid biosynthesis pathway repression

The transcription of amino acid biosynthesis genes is often induced by a low intracellular concentration.

Since intracellular concentrations cannot be determined by FBA, use fluxes to approximate the regulation.

Metabolite *H* represents the amino acid, and can be made by the cell via reaction *R8a* or transported from the extracellular media through transport process *Th*.

For the regulatory structure, *Th* will be used to activate *RPh* which will repress transcription of the gene encoding *R8a*.

$$RPh = IF\ (v_{Th} > 0),$$
$$tR8a = IF\ NOT\ (RPh).$$

Maintain concentrations

Transcriptional regulation maintains concentration levels of important metabolites.

The activation or repression of these genes depends on the level of *B* in the cell.

Use a flux rather than concentration to turn off an enzyme.

Choose *R2b* as the determining factor; it will activate *RPb* which in turn will inactivate *tR2a* and *tR7*.

$$RPb = IF\ (v_{R2b} > 0),$$
$$tR2a = IF\ NOT\ (RPb),$$
$$tR7 = IF\ NOT\ (RPb).$$

Mathematical model II

Reaction	Name	Regulation
Metabolic reactions		
$-1\ A - 1\ ATP + 1\ B$	*R1*	
$-1\ B + 2\ ATP + 2\ NADH + 1\ C$	*R2a*	*IF NOT(RPb)*
$-1\ C - 2\ ATP - 2\ NADH + 1\ B$	*R2b*	
$-1\ B + 1\ F$	*R3*	
$-1\ C + 1\ G$	*R4*	
$-1\ G + 0.8\ C + 2\ NADH$	*R5a*	*IF NOT (RPO2)*
$-1\ G + 0.8\ C + 2\ NADH$	*R5b*	*IF RPO2*
$-1\ C + 2\ ATP + 3\ D$	*R6*	
$-1\ C - 4\ NADH + 3\ E$	*R7*	*IF NOT (RPb)*
$-1\ G - 1\ ATP - 2\ NADH + 1\ H$	*R8a*	*IF NOT (RPh)*
$+1\ G + 1\ ATP + 2\ NADH - 1\ H$	*R8b*	
$-1\ NADH - 1\ O2 + 1\ ATP$	*Rres*	*IF NOT (RPO2)*
Transport processes		
$-1\ Carbon1 + 1\ A$	*Tc1*	
$-1\ Carbon2 + 1\ A$	*Tc2*	*IF NOT(RPc1)*
$-1\ F_{ext} + 1\ F$	*Tf*	
$-1\ D + 1\ D_{ext}$	*Td*	
$-1\ E + 1\ E_{ext}$	*Te*	
$-1\ H_{ext} + 1\ H$	*Th*	
$-1\ Oxygen + 1\ O2$	*To2*	
Maintenance and growth processes		
$-1\ C - 1\ F - 1\ H - 10\ ATP + 1\ Biomass$	*Growth*	
Regulatory proteins		
	RPO2	*IF NOT(Oxygen)*
	RPc1	*IF Carbon1*
	RPh	$IF\ (v_{Th} > 0)$
	RPb	$IF\ (v_{R2b} > 0)$

Generating dynamic profiles (rFBA)

Covert/Schilling/Palsson 2001

Divide experimental time into small time steps Δ t.

At a given time point, use linear programming to identify an optimal metabolic flux distribution (by maximizing the *Growth* flux).

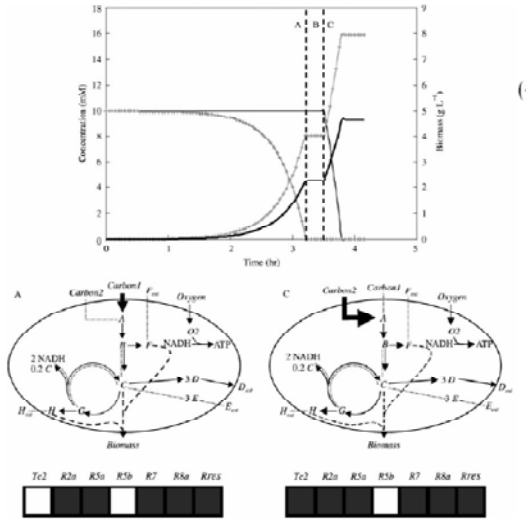
Using the resulting flux distribution and the conditions of the system in a previous time step, the conditions of the next time step are calculated to obtain biomass as well as extracellular substrate and by-product concentrations.

Numerical parameter values

Parameter	Value
Maximum transport rates (mmol g-DCW^{-1} hr^{-1})	
Carbon1	10.5
Carbon2	10.5
D	12.0
E	12.0
F	5.0
H	5.0
O2	15.0
Protein synthesis/decay delay (hr)	0.25

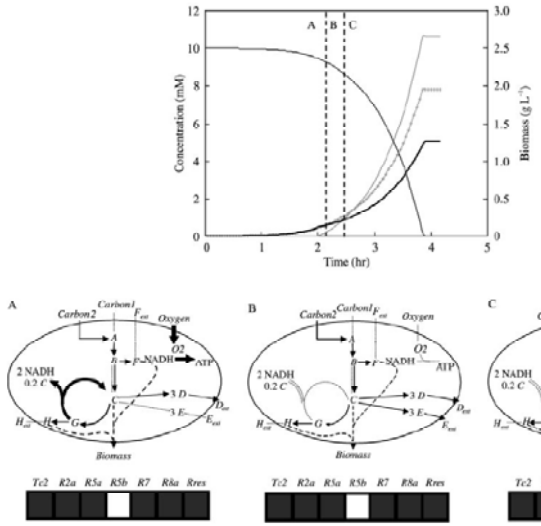
➡ five simulations to illustrate each regulatory element separately and in a complex medium

1. Catabolite repression



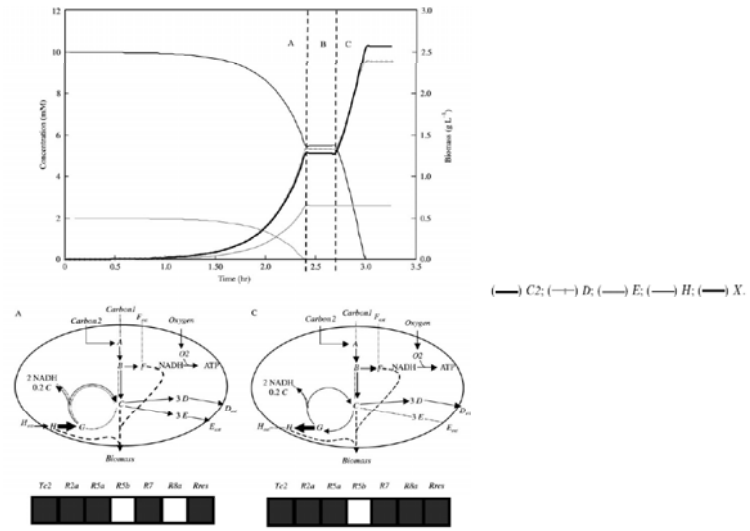
(—·—) *C1*; (——) *C2*; (—·—) *D*; (▬) *X*.

2. Aerobic/anaerobic diauxie

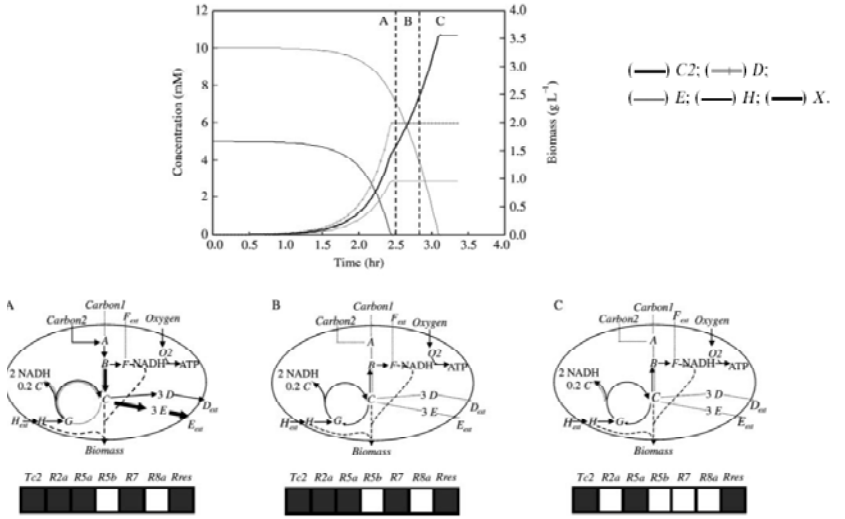


(——) *E*; (——) *C2*; (—·—) *D*; (▬) *X*.

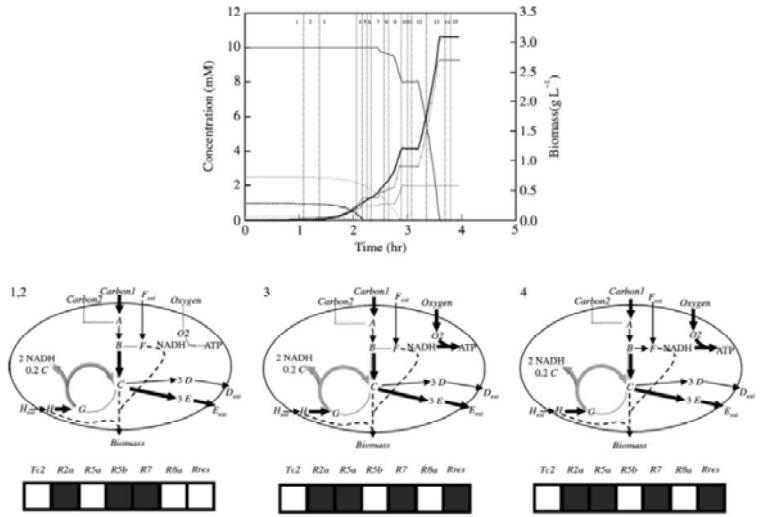
3. Amino acid biosynthesis



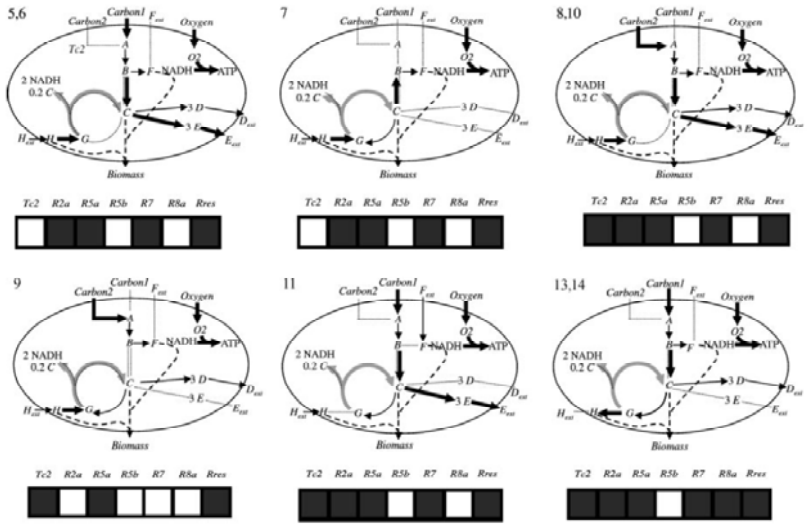
4. Growth on carbon and amino acid



5. Growth on complex media



5. Growth on complex media (ctd)



Discussion

1. quantitative dynamic simulation of substrate uptake, cell growth and by-product secretion;

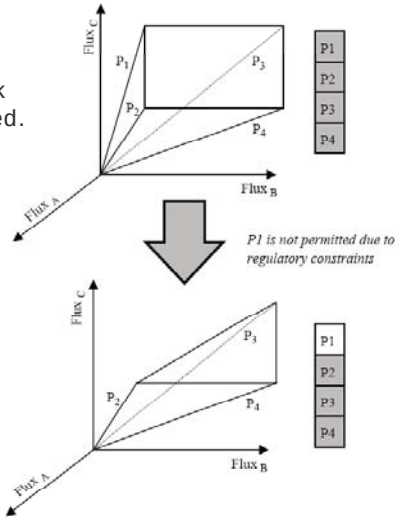
2. qualitative simulation of gene transcription events and the presence of proteins in the cell;

3. investigation of the systemic effects of imposing temporary regulatory constraints on the solution space.

Regulatory constraints and extreme pathways

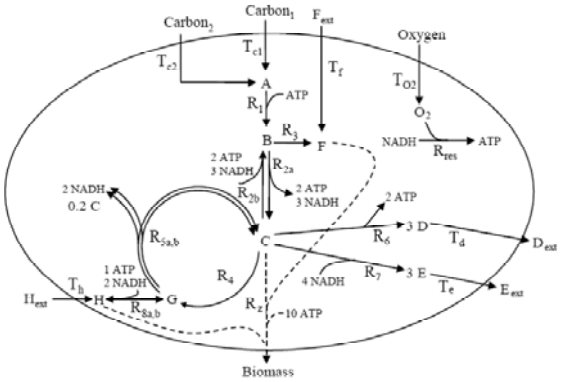
Covert/Palsson 2003

- Split all internal reversible reactions in a metabolic network
  ⇒ flux cone C becomes pointed.
- Extreme rays of C are called extreme pathways.
- Certain extreme pathways may not be permitted due to regulatory or environmental constraints.



Simplified core carbon metabolism

Covert/Schilling/Palsson 2001



REACTION	NAME	REGULATION
Metabolic Reactions		
A + ATP → B	R1	
B → 2 ATP + 2 NADH + C	R2a	IF NOT(RPb)
C + 2 ATP + 2 NADH → B	R2b	
B → F	R3	
C → G	R4	
G → 0.8 C + 2 NADH	R5a	IF NOT (RPo2)
G → 0.8 C + 2 NADH	R5b	IF RPo2
C → 2 ATP + 3 D	R6	
C + 4 NADH → 3 E	R7	IF NOT (RPb)
G + ATP + 2 NADH → H	R8a	IF NOT (RPh)
H → G + ATP + 2 NADH	R8b	
NADH + O2 → ATP	Rres	IF NOT (RPo2)
Transport Processes		
Carbon1 → A	Tc1	
Carbon2 → A	Tc2	IF NOT(RPc1)
Fext → F	Tf	
D → Dext	Td	
E → Eext	Te	
Hext → H	Th	
Oxygen → O2	To2	
Maintenance and Growth Processes		
C + F + H + 10 ATP → Biomass	Growth	
Regulatory Proteins		
	RPo2	IF NOT(Oxygen)
	RPc1	IF Carbon1
	RPh	IF Th
	RPb	IFR2b

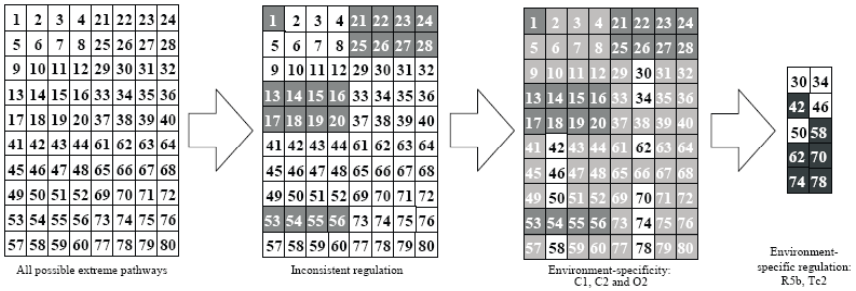
⇒ 80 extreme pathways (if neglecting regulatory constraints)

Impact of environment

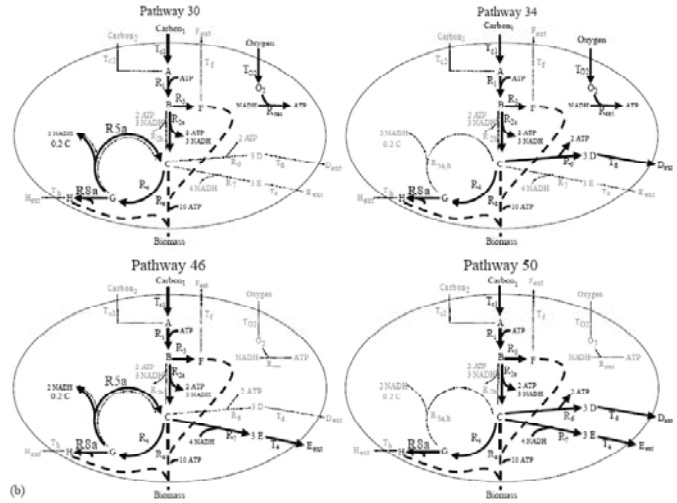
- 2^5 = 32 possible environments
- 21 extreme pathways always impossible due to regulatory constraints
- several environments show (near-)identical sets of extreme pathways.
- highest number of available pathways is 26, lowest number is 2.

Environments					Repressed enzymes					Pathways	Pathway list
C1	C2	F	H	O2			R_{5b}	R_{8a}	T_{c2}	26	P2, P4, P5, P6, P8, P9, P10, P12, P29, P30, P31, P32, P33, P34, P35, P36, P37, P38, P45, P46, P47, P48, P49, P50, P51, P52 Detail in Fig. 4.
C1	C2	F	H			R_{5a}		R_{8a}	R_{res} T_{c2}	10	P39, P40, P41, P42, P43, P44, P49, P50, P51, P52
C1	C2	F		O2			R_{5b}		T_{c2}	8	P29, P30, P33, P34, P45, P46, P49, P50
C1	C2	F				R_{5a}			R_{res} T_{c2}	4	P41, P42, P49, P50
C1	C2		H	O2			R_{5b}	R_{8a}	T_{c2}	14	P2, P5, P6, P9, P10, P30, P31, P34, P35, P37, P46, P47, P50, P51
C1	C2		H			R_{5a}		R_{8a}	R_{res} T_{c2}	5	P39, P42, P43, P50, P51
C1	C2			O2			R_{5b}		T_{c2}	4	P30, P34, P46, P50 Detail in Fig. 3
C1	C2					R_{5a}			R_{res} T_{c2}	2	P42, P50
C1		F	H	O2			R_{5b}	R_{8a}	T_{c2}	26	P2, P4, P5, P6, P8, P9, P10, P12, P29, P30, P31, P32, P33, P34, P35, P36, P37, P38, P45, P46, P47, P48, P49, P50, P51, P52
C1		F	H			R_{5a}		R_{8a}	R_{res} T_{c2}	10	P39, P40, P41, P42, P43, P44, P49, P50, P51, P52
C1		F		O2			R_{5b}		T_{c2}	8	P29, P30, P33, P34, P45, P46, P49, P50
C1		F				R_{5a}			R_{res} T_{c2}	4	P41, P42, P49, P50
C1			H	O2			R_{5b}	R_{8a}	T_{c2}	14	P2, P5, P6, P9, P10, P30, P31, P34, P35, P37, P46, P47, P50, P51
C1			H			R_{5a}		R_{8a}	R_{res} T_{c2}	5	P39, P42, P43, P50, P51
C1				O2			R_{5b}		T_{c2}	4	P30, P34, P46, P50
C1						R_{5a}			R_{res} T_{c2}	2	P42, P50
	C2	F	H	O2			R_{5b}	R_{8a}		26	P3, P4, P5, P7, P8, P9, P11, P12, P57, P58, P59, P60, P61, P62, P63, P64, P65, P66, P73, P74, P75, P76, P77, P78, P79, P80
	C2	F	H			R_{5a}		R_{8a}	R_{res}	10	P67, P68, P69, P70, P71, P72, P77, P78, P79, P80
	C2	F		O2			R_{5b}			8	P57, P58, P61, P62, P73, P74, P77, P78
	C2	F				R_{5a}			R_{res}	4	P69, P70, P77, P78
	C2		H	O2			R_{5b}	R_{8a}		14	P3, P5, P7, P9, P11, P58, P59, P62, P63, P65, P74, P75, P78, P79
	C2		H			R_{5a}		R_{8a}	R_{res}	5	P67, P70, P71, P78, P79
	C2			O2			R_{5b}			4	P58, P62, P74, P78
	C2					R_{5a}			R_{res}	2	P70, P78
		F	H	O2			R_{5b}	R_{8a}		5	P4, P5, P8, P9, P12
		F	H		R_{2a}	R_{5a}		R_{8a}	R_{res}	0	
		F		O2			R_{5b}			0	
		F				R_{5a}			R_{res}	0	
			H	O2	R_{2a}		R_{5b}	R_{8a}		2	P5, P9
			H		R_{2a}	R_{5a}		R_{8a}	R_{res}	0	
				O2			R_{5b}			0	

Growth on C1, C2 and O2



Four remaining extreme pathways



Growth on C1, C2, F, H and O2

P#																	
8				0.283	Fext	+	0.717	Hext	+	2.391	Oxygen					0.283	Biomass
29	0.717	Carbon1	+	0.283	Fext				+	2.391	Oxygen					0.283	Biomass
32	0.435	Carbon1	+	0.283	Fext	+	0.283	Hext	+	2.391	Oxygen					0.283	Biomass
5							1.000	Hext	+	2.596	Oxygen					0.277	Biomass
6	0.277	Carbon1				+	0.723	Hext	+	2.596	Oxygen					0.277	Biomass
30	1.000	Carbon1							+	2.596	Oxygen					0.277	Biomass
31	0.723	Carbon1				+	0.277	Hext	+	2.596	Oxygen					0.277	Biomass
9							1.000	Hext	+	2.390	Oxygen	0.146	Dext		+ 0.268	Biomass	
12				0.262	Fext	+	0.738	Hext	+	1.905	Oxygen	0.357	Dext		+ 0.262	Biomass	
10	0.252	Carbon1				+	0.748	Hext	+	1.985	Oxygen	0.435	Dext		+ 0.252	Biomass	
33	0.773	Carbon1	+	0.227	Fext				+	1.091	Oxygen	0.955	Dext		+ 0.227	Biomass	
36	0.545	Carbon1	+	0.227	Fext	+	0.227	Hext	+	1.091	Oxygen	0.955	Dext		+ 0.227	Biomass	
34	1.000	Carbon1							+	1.130	Oxygen	1.043	Dext		+ 0.217	Biomass	
35	0.783	Carbon1				+	0.217	Hext	+	1.130	Oxygen	1.043	Dext		+ 0.217	Biomass	
4				0.188	Fext	+	0.813	Hext	+	1.250	Oxygen			0.938	Eext	+ 0.188	Biomass
2	0.170	Carbon1				+	0.830	Hext	+	1.208	Oxygen			1.075	Eext	+ 0.170	Biomass
49	0.875	Carbon1	+	0.125	Fext							0.750	Dext	+ 1.125	Eext	+ 0.125	Biomass
52	0.750	Carbon1	+	0.125	Fext	+	0.125	Hext				0.750	Dext	+ 1.125	Eext	+ 0.125	Biomass
50	1.000	Carbon1										0.794	Dext	+ 1.147	Eext	+ 0.118	Biomass
51	0.882	Carbon1				+	0.118	Hext				0.794	Dext	+ 1.147	Eext	+ 0.118	Biomass
38	0.357	Carbon1	+	0.083	Fext	+	0.560	Hext						+ 1.964	Eext	+ 0.083	Biomass
45	0.917	Carbon1	+	0.083	Fext									+ 1.964	Eext	+ 0.083	Biomass
48	0.833	Carbon1	+	0.083	Fext	+	0.083	Hext						+ 1.964	Eext	+ 0.083	Biomass
37	0.429	Carbon1				+	0.571	Hext						+ 2.011	Eext	+ 0.077	Biomass
46	1.000	Carbon1												+ 2.011	Eext	+ 0.077	Biomass
47	0.923	Carbon1				+	0.077	Hext						+ 2.011	Eext	+ 0.077	Biomass

Group 1 Group 2

D E

- 26 extreme pathways, four groups with high similarity
- small degree of variation, once regulatory constraints are taken into account.

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