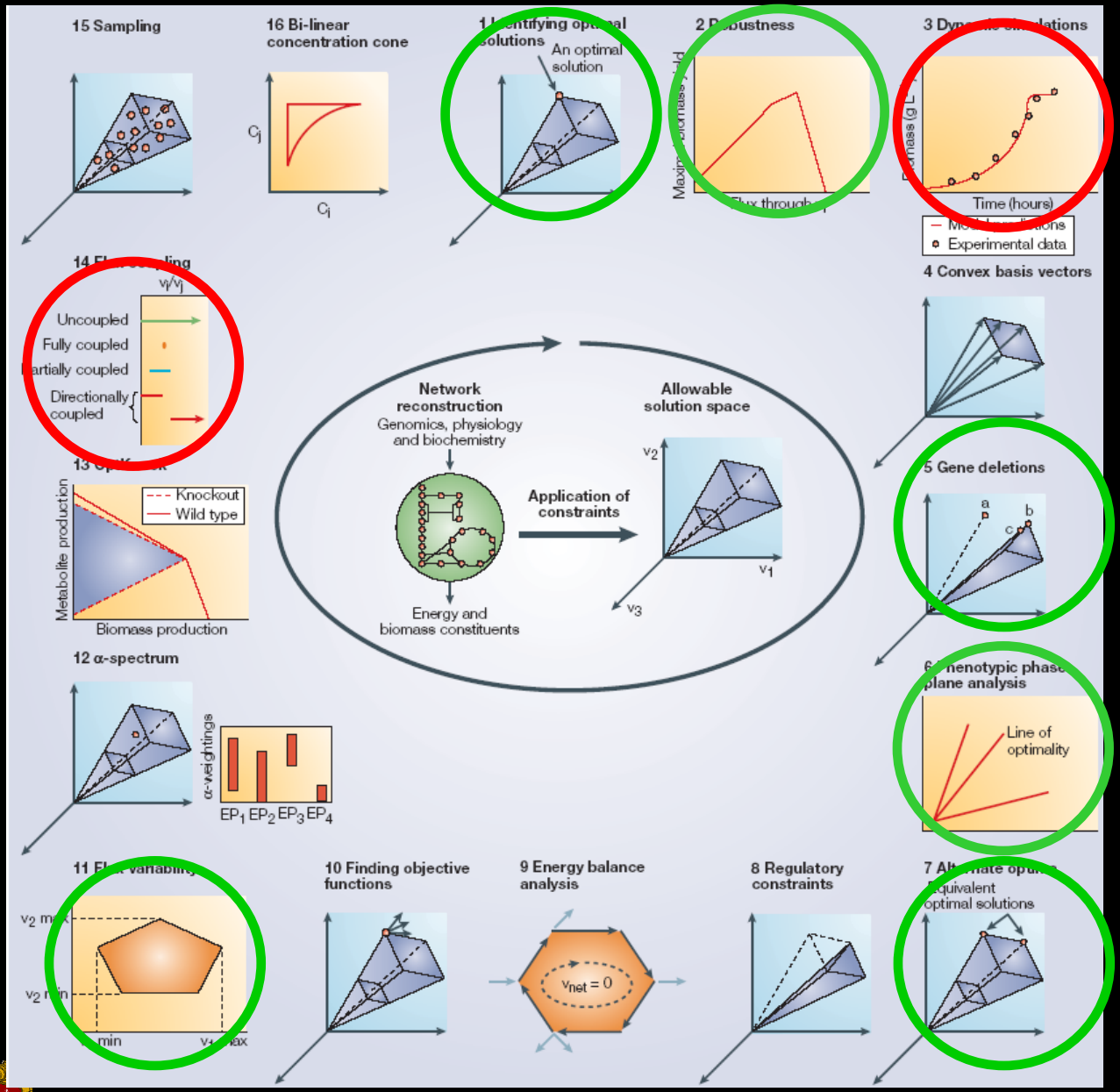
Constraint-Based Workshops

8. Batch Culture & Flux Coupling
February 18th, 2008

Constraint-Based Methods

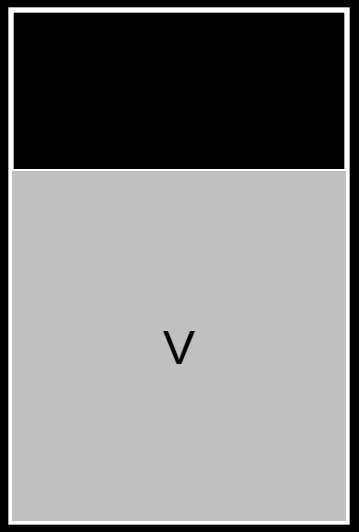


- Optimal Solutions
  1. FBA
  2. Flux Variability
- Flux Dependencies
  1. Robustness
  2. Phase Planes
  3. Flux Coupling
- All Allowable Solutions
  1. Extreme Pathways
  2. Elementary Modes
  3. Sampling
- Altering Phenotypes
  1. Genetic Mutations
  2. Strain Design
- Application of Additional Constraints
  1. Regulation
  2. Energy Balance

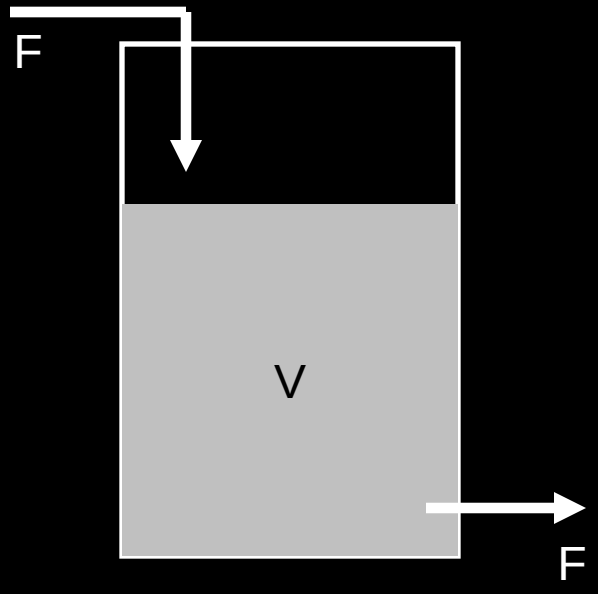
Price, Reed, and Palsson
Nat. Reviews Microbiol.
2004

Overview of Cellular Growth

- Batch
  - Multiple growth phases, exponential growth has constant growth rate, μ.



- CSTR or Bioreactor
  - @ SS: All concentrations are constant.



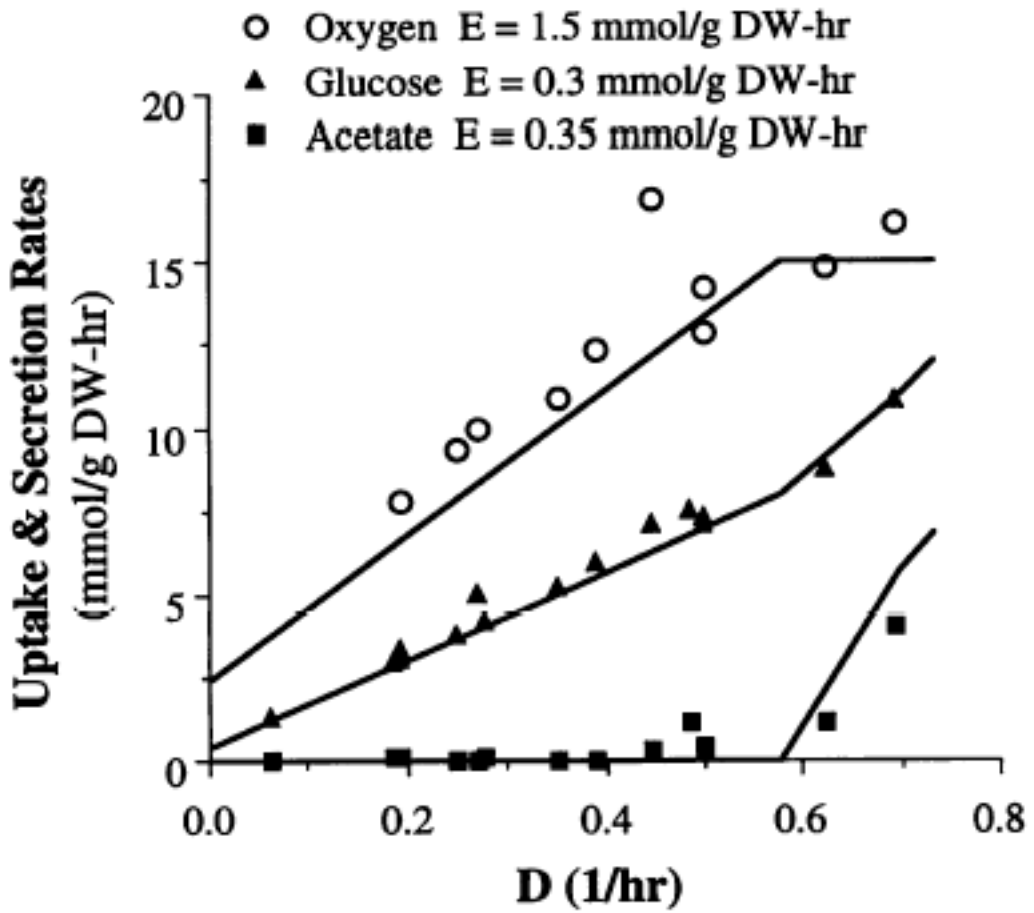
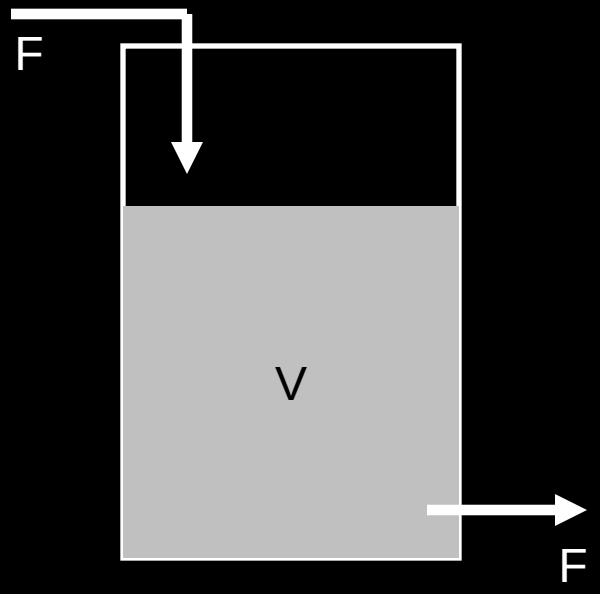


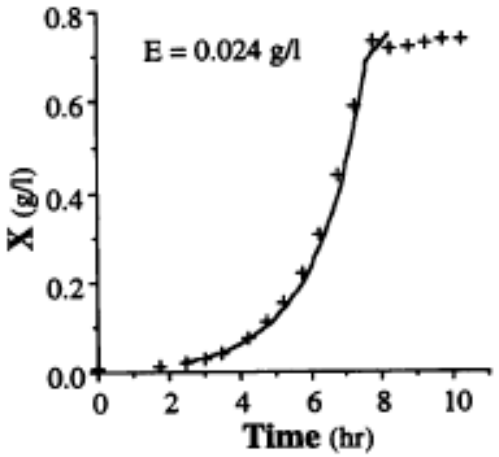
FIG. 6. Analysis of aerobic chemostat culture showing the glucose and oxygen uptake rates and the acetate secretion rate as functions of the dilution or growth rate. The chemostat was not limited for minerals. The solid lines represent the flux balance model simulations. E, average deviation between predictions of the model and experimental measurements; DW, dry weight.

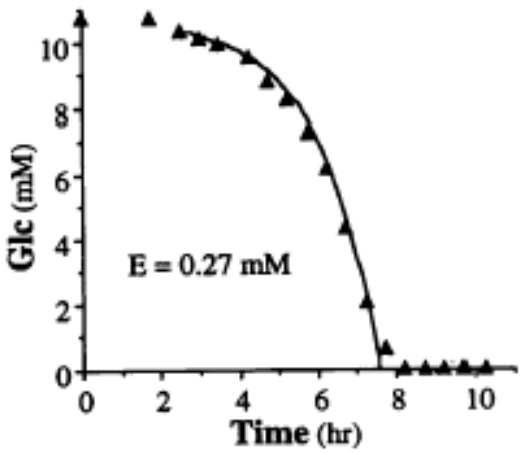
In Chemostat:

D = F/V = μ @ SS



Varma and Palsson, App. Environ. Microbiol. 60(10): 3723-3731 (1994)





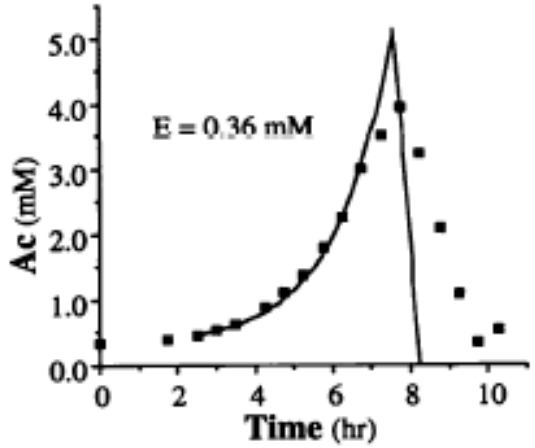


FIG. 7. Analysis of aerobic batch culture showing time profiles of cell density, glucose concentration, and acetate (Ac) concentration. The culture was not limited for minerals. The solid lines are the flux balance model predictions of the time profiles for the culture. E, average deviation between predictions of the model and experimental measurements.

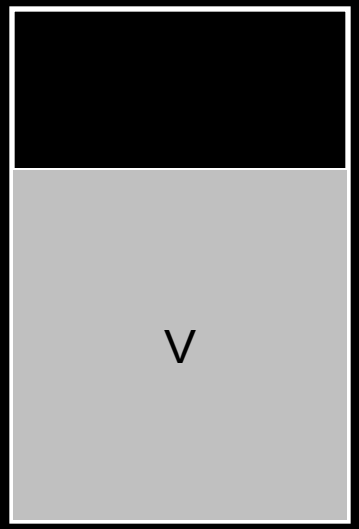
<u>In Batch Culture:</u>

V is Constant

Biomass (X) increases over time

Carbon Source (eg. glucose) decreases over time

*Acetate is secreted if not enough Oxygen is supplied



Varma and Palsson, App. Environ. Microbiol. 60(10): 3723-3731 (1994)

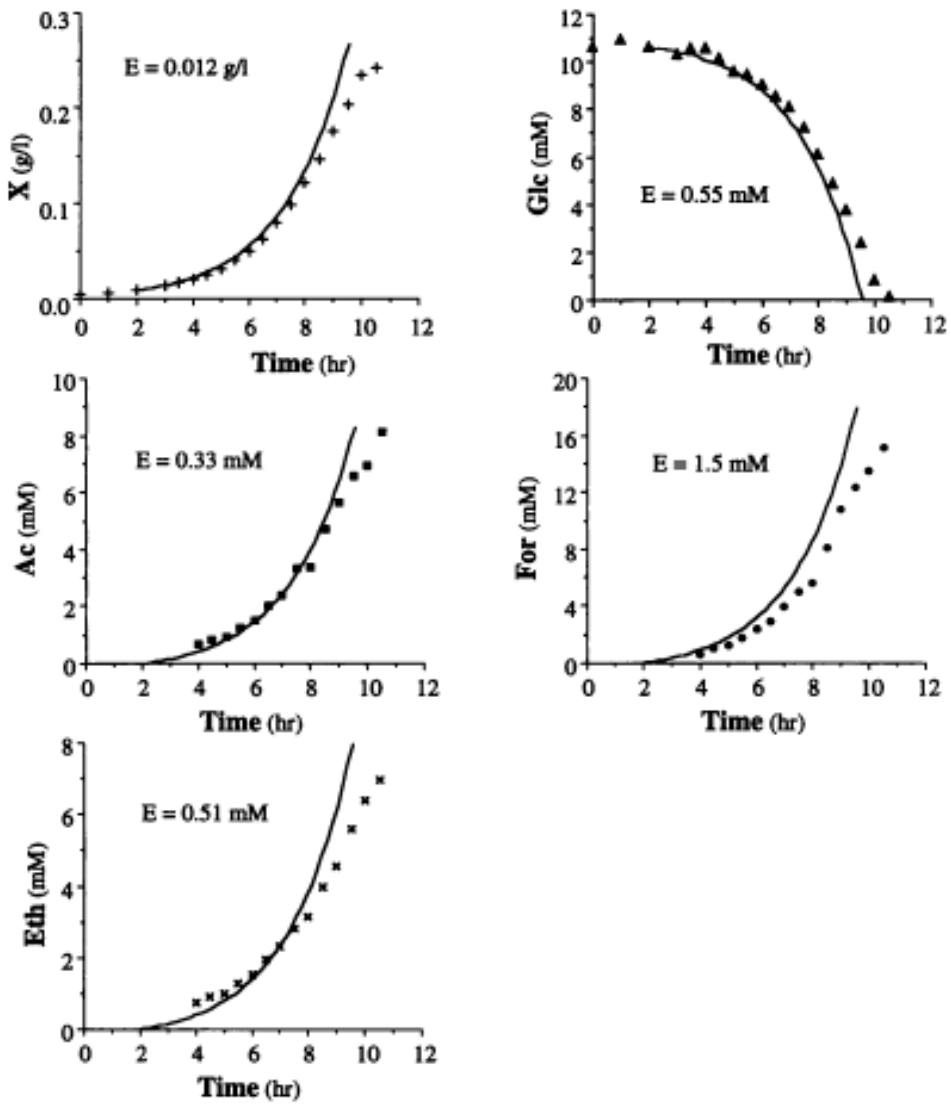


FIG. 11. Anaerobic batch culture showing the time profiles of cell density and various by-product concentrations. Solid lines represent the model predictions of the model. E, average deviation between predictions of the model and experimental measurements; Ac, acetate; For, formate; Eth, ethanol.

In Batch Culture:

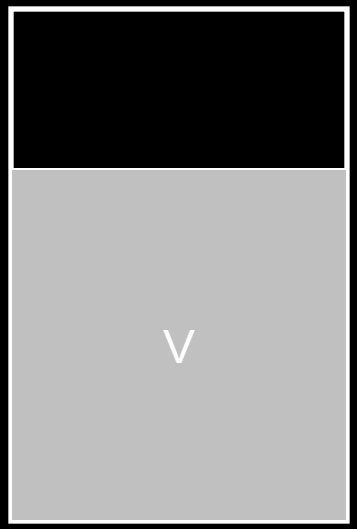
V is Constant

Biomass (X) increases over time

Carbon Source (eg. glucose) decreases over time

*Acetate and other by-products are secreted to consume NADH made during glycolysis

(No Oxygen means No Respiration)



Varma and Palsson, App. Environ. Microbiol. 60(10): 3723-3731 (1994)

How to Approximate Batch Simulations?

- During each <u>small</u> time step, Δt, we will assume that uptake & secretion rates and μ are constant.

$X(t)$ = biomass concentration
$S(t)$ = substrate concentration
$P(t)$ = product concentration
v has units of mmol/gDW/hr

$$\begin{aligned} dX/dt &= \mu X \\ dS/dt &= v_{EX\_S} \cdot X \\ dP/dt &= v_{EX\_P} \cdot X \end{aligned} \quad \longrightarrow \quad \begin{aligned} X(t+\Delta t) &= X(t) \cdot e^{\mu \Delta t} \\ S(t+\Delta t) &= S(t) - v_{EX\_S} \cdot X(t) \cdot (1-e^{\mu \Delta t})/\mu \\ P(t+\Delta t) &= P(t) - v_{EX\_P} \cdot X(t) \cdot (1-e^{\mu \Delta t})/\mu \end{aligned}$$

We need to know v_{EX_S}, v_{EX_P}, μ!
(we can use FBA to calculate these)

Constraining Exchange Fluxes:

- *During each <u>small</u> time step, Δt, the substrate uptake rate depends on the current substrate and cell concentration and the cells maximum uptake ability:*

$$v_{EX\_S\_available} = - S(t) / [X(t) \cdot \Delta t]$$

For example: if there is 0.2mmol glucose/L and 1 gDW cell/L, then in 0.1 hrs the most substrate you could take up is 2mmol/gDW/hr.

This might exceed the cells uptake capacity, so you take either the cell's capacity or the availability as the limit.

Maximum Uptake Rate = $\max(v_{EX\_S\_available}, v_{EX\_S\_max})$
ie. which ever is less negative

An Illustrative Example:

- During aerobic growth the maximum cellular uptake for glucose is 10.5 mmol/gDW/hr (18.5 for anaerobic growth) and the maximum O_2 uptake is 15.

- At t=2 hours, [glucose] = 10.9 mmol/L and X=0.08 gDW/L. If our Δt is 0.1 hrs, then:

  $v_{EX\_glc\_avail}$ = -136.

- This exceeds our cell capacity ($v_{EX\_glc\_max}$) of -10.5.
- We would run FBA with a lower glucose uptake rate of -10.5mmol/gDW/hr

- At t=9 hours, [glucose] = 0.5 mmol/L and X=0.7 gDW/L. If our Δt is 0.1 hrs, then:

  $v_{EX\_glc\_avail}$ = -7.1.

- Our cell capacity ($v_{EX\_glc\_max}$) of -10.5 exceeds what is available in the media.
- We would run FBA with a lower glucose uptake rate of -7.1mmol/gDW/hr

```
*allow co2,pi,o2,h,h2o to be taken up by the cell
LowerLimits('EX_co2_e')=-Vmax;
LowerLimits('EX_h2o_e')=-Vmax;
LowerLimits('EX_h_e')=-Vmax;
LowerLimits('EX_pi_e')=-Vmax;
UpperLimits('ATPM')=7.6;
LowerLimits('ATPM')=7.6;
UpperLimits('EX_glc_e')=0;

*Scaling Factor Introduced By Varma and Palsson AEM 1994
S(i,'Biomass')=1.3*S(i,'Biomass');

sets timesteps /time1*time121/
media /biomass,glucose,acetate,form,lactate,ethanol,succinate/;

Parameter
c(j) used to define the objective function for FBA
time(timesteps)
oxygenuptake  mmol per gDW per hour (max is 15) /15/
glucoseuptake mmol per gDW per hour (max is 10.5 aero
acetateuptake mmol per gDW per hour /3.1/
initialglucose mmol per liter /11/
initialacetate mmol per liter /0/
initialbiomass gDW per liter /0.002/
dt units of hours /0.1/
concentrations(timesteps,media) mmol per liter;

c('Biomass')=1;
LowerLimits('EX_o2_e')=-oxygenuptake;

Variables
v(j) flux values through reaction in network
Obj  this is the value of the objective function for the FBA solutions;
```

ATP Maintenance Flux = 7.6mmol/gDW/hr

Scaling Factor

Total Time = (121-1)/dt = 12 hours

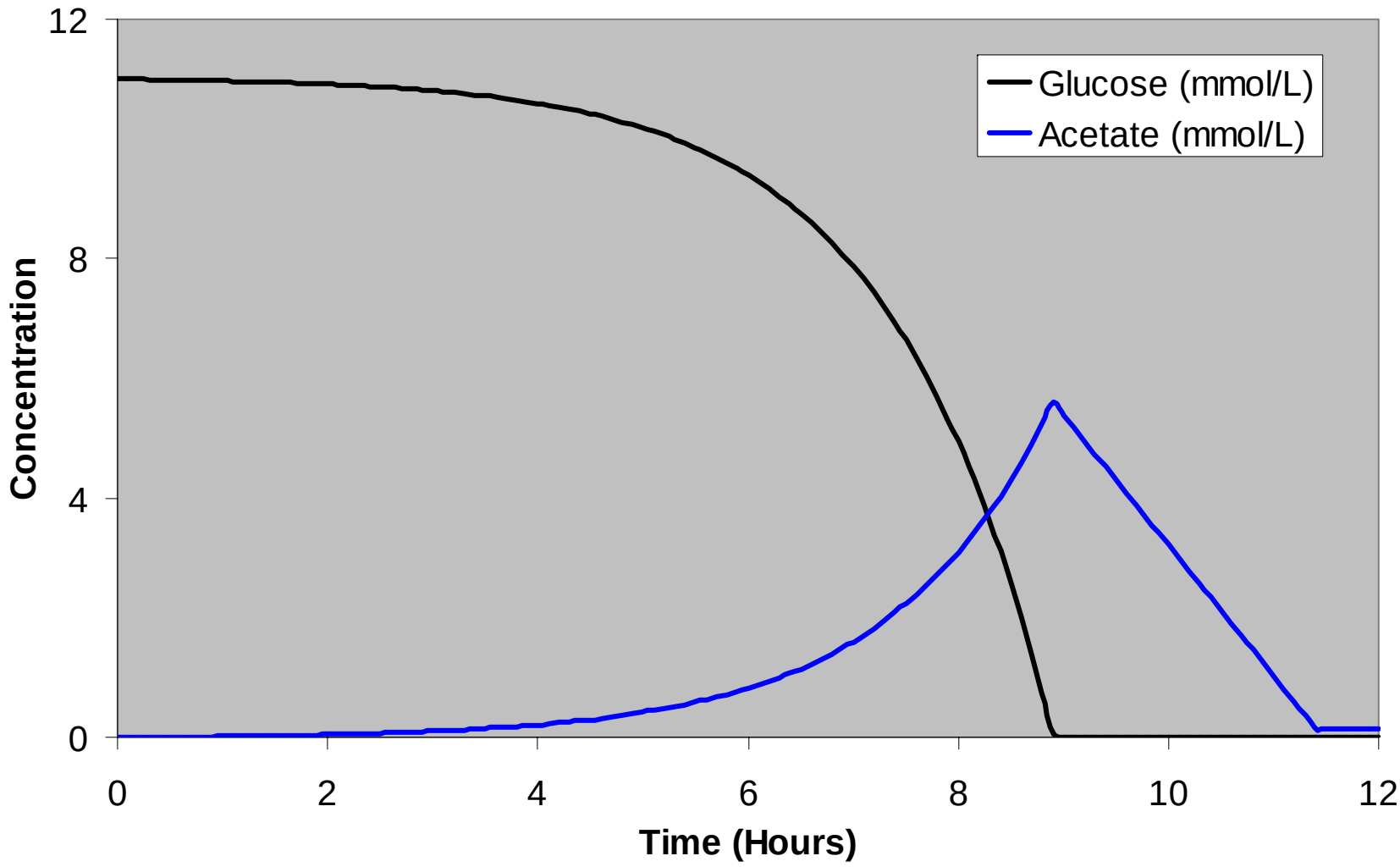
Cellular Uptake Capacity (note - signs introduced later on below for EX fluxes)

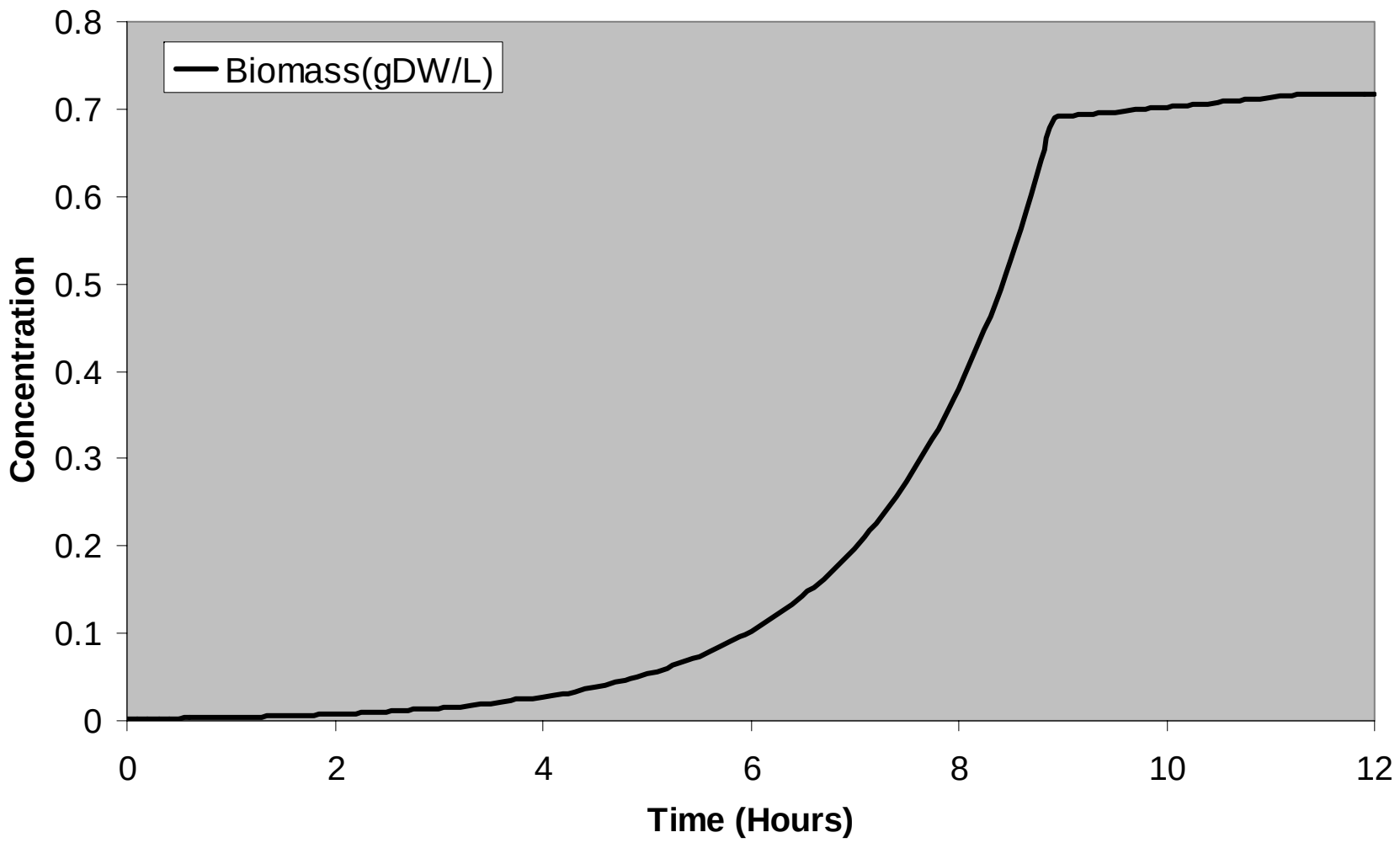
Concentrations at t=0

Time Step

Batch Calculations: Aerobic

- Graph concentrations versus time for glucose aerobic growth.
- When does glucose run out? When is acetate reconsumed?
- What are the final yields before acetate is reconsumed:
  - Biomass: $-\Delta X/\Delta Glc$ (gDW cells/mmol glucose)
  - Acetate: $-\Delta Ac/\Delta Glc$ (mmol acetate/mmol glucose)


12
8
4
0
Concentration
0
2
4
6
8
10
12
Time (Hours)
Glucose (mmol/L)
Acetate (mmol/L)

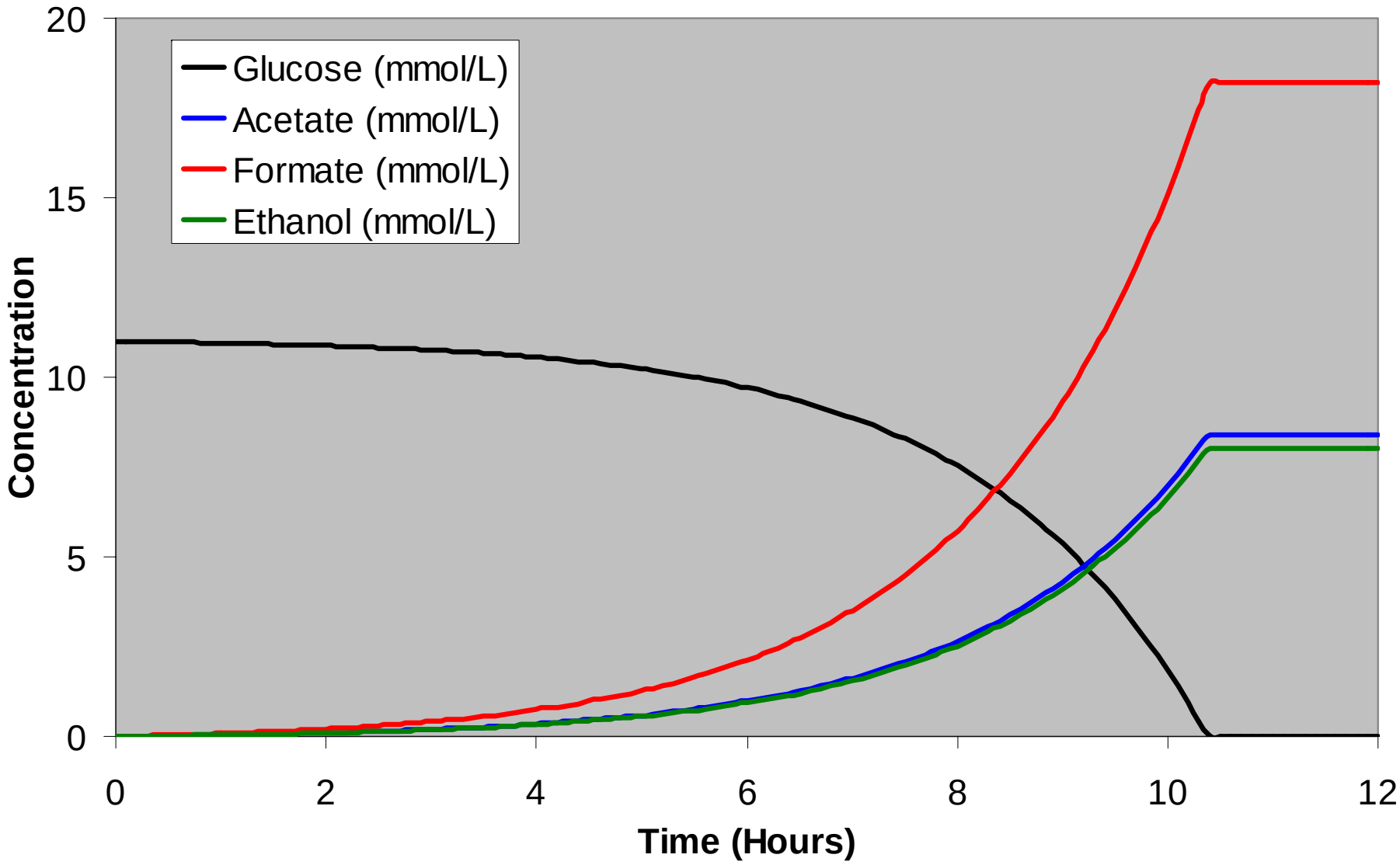

Biomass(gDW/L)
Concentration
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
2
4
6
8
10
12
Time (Hours)

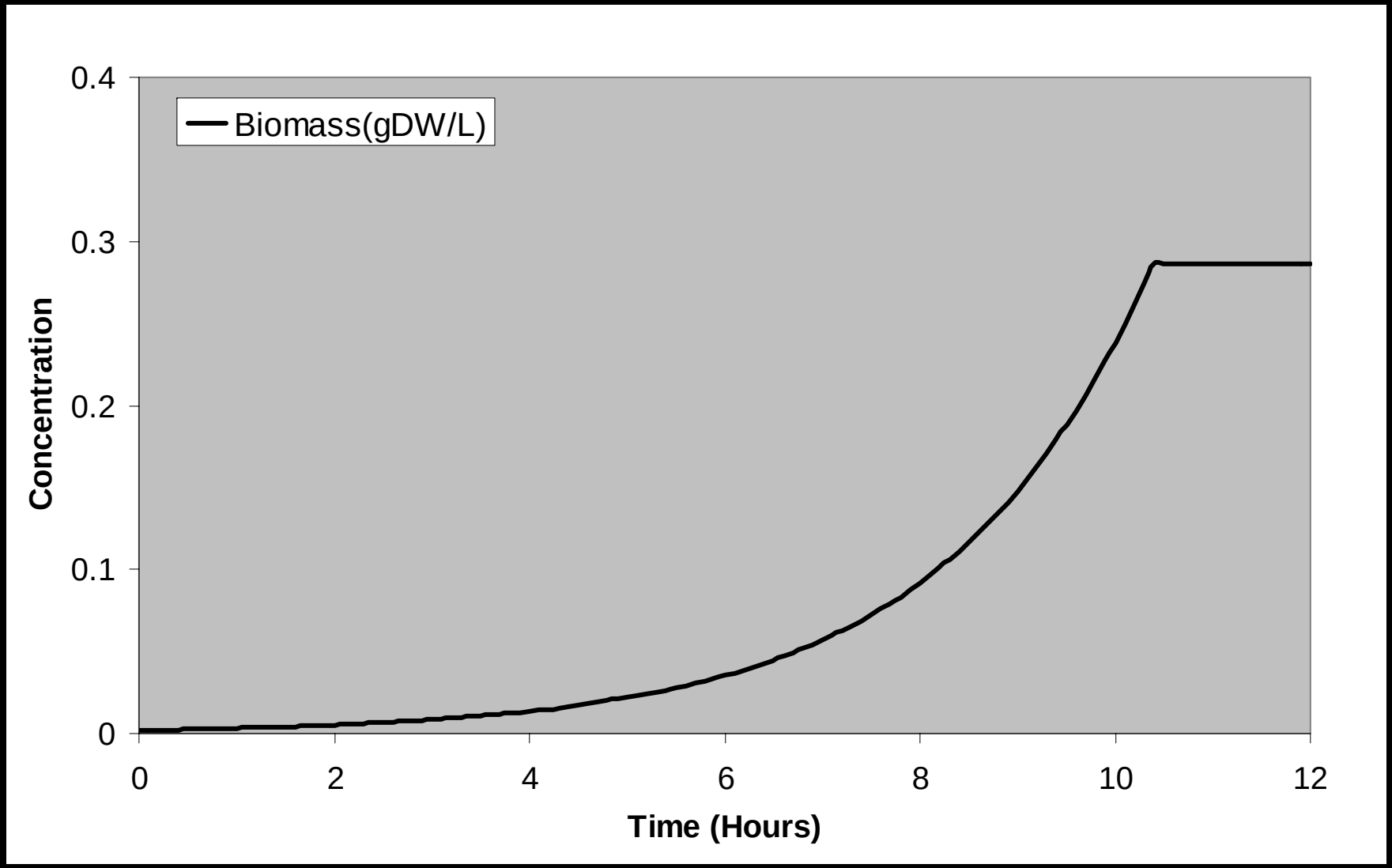
Batch Calculations: Aerobic

- When does glucose run out? When is acetate reconsumed?
  - ANS: At 9 hours
- What are the final yields before acetate is reconsumed:
  - Biomass: 0.063 g/mmol ~ 0.35 g/g
  - Acetate: 0.509 mmol/mmol ~ 0.16 g/g

Batch Calculations: Anaerobic

- Graph concentrations versus time for glucose anaerobic growth.
- What are the final yields:
  - Biomass: $-\Delta X/\Delta Glc$ (gDW cells/mmol glucose)
  - Acetate: $-\Delta Ac/\Delta Glc$ (mmol acetate/mmol glucose)
  - Ethanol: $-\Delta Etoh/\Delta Glc$ (mmol ethanol/mmol glucose)
  - Lactate: $-\Delta Lac/\Delta Glc$ (mmol lactate/mmol glucose)
- If you start out with more cells initially (0.02 g/L) does this speed up or slow down the time course? Does the Biomass yield change?


Glucose (mmol/L)
Acetate (mmol/L)
Formate (mmol/L)
Ethanol (mmol/L)
Concentration
20
15
10
5
0
0
2
4
6
8
10
12
Time (Hours)


Biomass(gDW/L)
0.4
0.3
0.2
0.1
0
Concentration
0
2
4
6
8
10
12
Time (Hours)

Batch Calculations: Anaerobic

- Graph concentrations versus time for glucose anaerobic growth.
- What are the final yields :
  - Biomass: 0.026 gDW/mmol ~0.14g/g
  - Acetate: 0.76 mmol/mmol ~ 0.25g/g
  - Formate: 1.65 mmol/mmol ~0.41g/g
  - Ethanol: 0.73 mmol/mmol ~0.19g/g
- If you start out with more cells (0.02 g/L) initially does this speed up or slow down the time course? Does the biomass yield change?
  - Now growth stops at 5.7 hours instead of 10.4
  - Biomass yield is still 0.026 gDW/mmol

Flux Coupling

- Used to see how pairs of fluxes affect one another.
- Done by calculating the minimum and maximum ratio between two fluxes
- Transformation needed to make it a linear problem

$$\begin{aligned}
&\text{maximize (or minimize)} && v_1/v_2 && \\
&\text{subject to} && \sum_{j=1}^{M} S_{ij} v_j = 0, && \forall\, i \in \mathrm{N} \\
& && v_j^{uptake} \le v_j^{uptake_max}, && \forall\, j \in \mathrm{M}_{\mathrm{transport}} \\
& && v_j \ge 0, && \forall\, j \in \mathrm{M}
\end{aligned}$$

$$\begin{aligned}
&\text{maximize or (minimize)} && \hat{v}_1 && (\mathrm{P}') \\
&\text{subject to} && \hat{v}_2 = 1 && \\
& && \sum_{j=1}^{M} S_{ij} \hat{v}_j = 0, && \forall\, i \in \mathrm{N} \\
& && \hat{v}_j^{uptake} \le v_j^{uptake_max} \cdot t, && \forall\, j \in \mathrm{M}_{\mathrm{transport}} \\
& && \hat{v}_j \ge 0, && \forall\, j \in \mathrm{M} \\
& && t \ge 0 &&
\end{aligned}$$

Burgard, AP, et al. Genome Research. 14(2):301-12 (2004).

Types of Coupling:

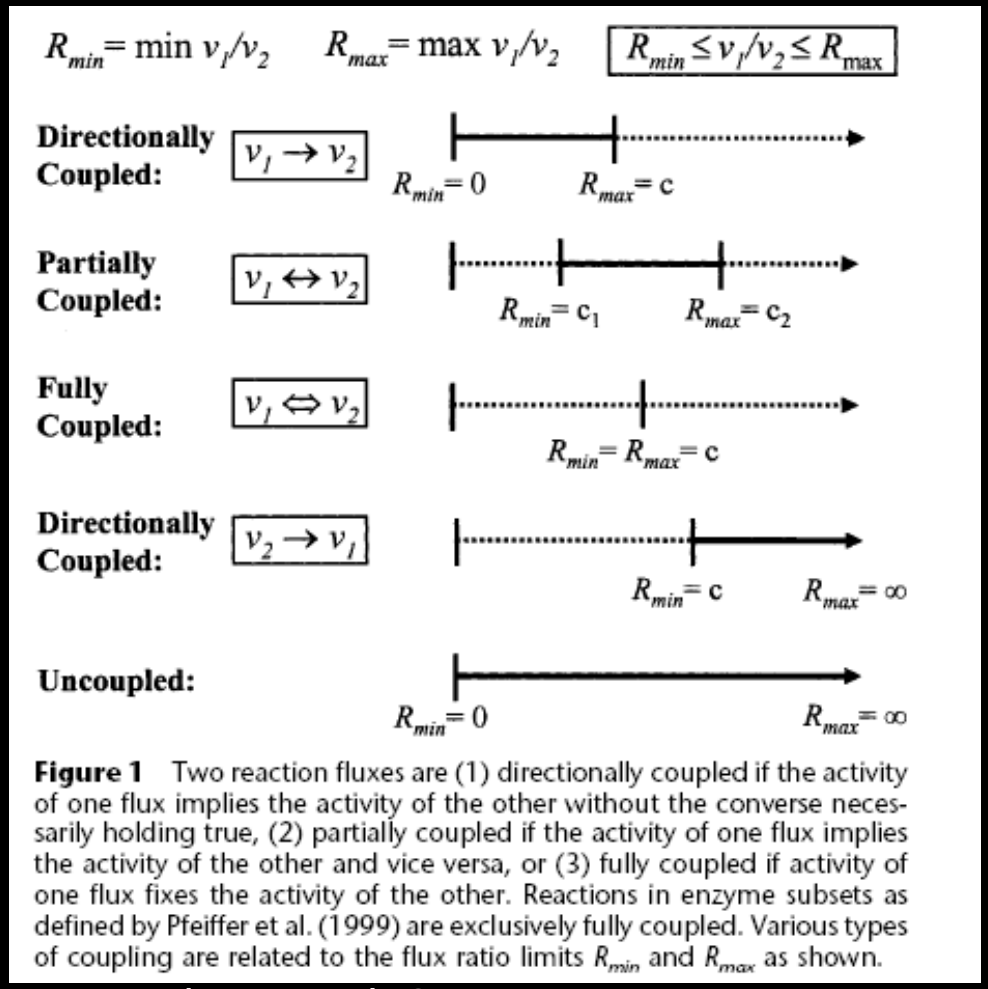
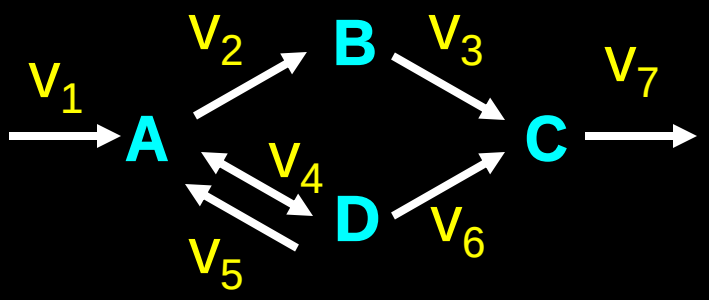


Figure 1 Two reaction fluxes are (1) directionally coupled if the activity of one flux implies the activity of the other without the converse necessarily holding true, (2) partially coupled if the activity of one flux implies the activity of the other and vice versa, or (3) fully coupled if activity of one flux fixes the activity of the other. Reactions in enzyme subsets as defined by Pfeiffer et al. (1999) are exclusively fully coupled. Various types of coupling are related to the flux ratio limits R_{min} and R_{max} as shown.

Burgard, AP, et al. Genome Research. 14(2):301-12 (2004).



- Fully Coupled:
  - V1 and V7
  - V2 and V3
- Directionally Coupled:
  - V2,V3 → V1,V7
  - V6 → V1,V4,V7
  - V5 → V4
- Uncoupled:
  - V5 w/ all other fluxes, except V4
  - V4 w/ all other fluxes, except V5 and V6
  - V6 w/ V2, V3 and V5

```
Sets
irr(j) /v1,v2,v3,v5,v6,v7/
rev(j) /v4/;
```

Separate Irreversible & Reversible Reactions

```
Variables
v_irr(irr) irreversible fluxes
v_for(rev) reversible fluxes forward direction
v_back(rev) reversible fluxes backward direction
Obj this is the value of the objective function for the FBA solutions;
```

In S·v=0 equation split reversible reactions (v_for and v_back fluxes)

```
Equations
massbalance(i) mass balance equations for each metabolite
calcobj calculates the dot product of the c vector the flux vector;
massbalance(i).. sum(irr,S(i,irr)*v_irr(irr)) + sum(rev,S(i,rev)*(v_for(rev)-v_back(rev)))=e=0;
calcobj.. Obj=e=sum(irr,c_irr(irr)*v_irr(irr))+sum(rev,c_for(rev)*v_for(rev)+c_back(rev)*v_back(rev));

Model FBA /massbalance, calcobj/;

v_irr.lo(irr)=0;
v_irr.up(irr)=Vmax;
v_for.lo(rev)=0;
v_for.up(rev)=Vmax;
v_back.lo(rev)=0;
v_back.up(rev)=Vmax;
```

All Fluxes Constrained Between 0 and Vmax

```
*********************************************************************
*This Section Finds All Blocked Reactions That Need to Be Removed*
*********************************************************************
set unblocked_irr(j)
    totalunblocked(j);
unblocked_irr(j)=no;
alias(dummyindex,irr);
loop(dummyindex, c_irr(dummyindex)
        solve FBA using lp maximi
        if(Obj.l=0,unblocked_irr(
        else unblocked_irr(dummyi
        c_irr(dummyindex)=0; );
```

Flux Variability Analysis to find actual Vmax and Vmin for each flux →
This will allow us to remove blocked reactions (including infeasible directions of reversible reactions)

```
*****************************************************************
***This Section Calculates the Minimum and Maximum Flux Ratios****
*****************************************************************
Parameters
d(j)
d_back(j)
S_mod(i,j);

S_mod(i,j)=S(i,j);
S_mod(i,unblocked_backonly)=-S(i,unblocked_backonly);
unblocked_irr(j)=unblocked_irr(j)+unblocked_backonly(j);

Variables
v_hat_irr(j) flux values through reaction in network
v_hat_for(j) flux values through reaction in network
v_hat_back(j) flux values through reaction in network
Obj_hat
t;

v_hat_irr.lo(j)=0;
v_hat_for.lo(j)=0;
v_hat_back.lo(j)=0;
v_hat_irr.up(j)=inf;
v_hat_for.up(j)=inf;
v_hat_back.up(j)=inf;
t.lo=0;

Equations
massbalance_hat(i) mass balance equations for each metabolite
calcobj_hat calculates the dot product of the c vector the flux vector
uptakelimit;

massbalance_hat(i).. sum(unblocked_irr,S_mod(i,unblocked_irr)*v_hat_irr(unblocked_irr)) + sum(unblocked_both,S_mod(i,
calcobj_hat.. Obj_hat=e=sum(unblocked_irr,d(unblocked_irr)*v_hat_irr(unblocked_irr))+sum(unblocked_both,d(unblocked_b
uptakelimit.. v_hat_irr('v1')=l=t*10;
Model FluxCoupling /massbalance_hat,calcobj_hat,uptakelimit/;
```

All Flux Ratios (v_hat) Constrained Between 0 and Inf

For the uptake flux, add additional constraint allowing its ratio to vary

GAMS File Results

- Two Text Files (row I, column J = Ratio for V_J/V_I:
  - "OneDirection" reports the max and min ratios in tables for the irreversible reactions compared to all other fluxes (irreversible and reversible fluxes).
  - "BothDirection" reports the max and min ratios in tables for the reversible reactions with each other (note that reversibility is redefined after FVA is performed)

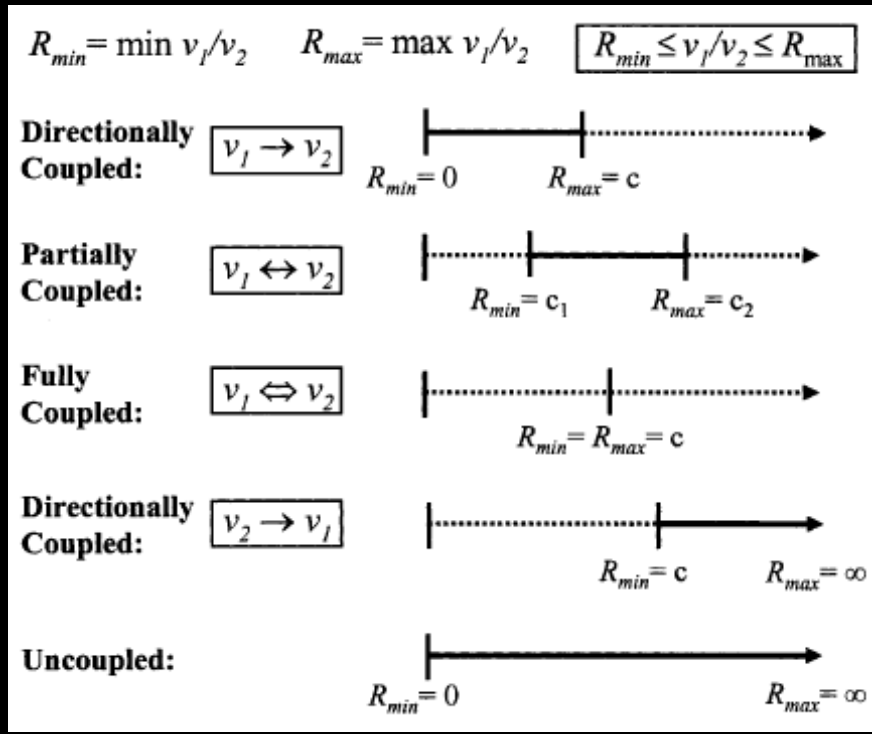
Results from the Example Network

FluxCouplingExample_OneDirection.txt - WordPad

File Edit View Insert Format Help

```
"MAXRATIO","v1","v2","v3","v4","v5","v6","v7"
"v1",1.0000,1.0000,1.0000,"+INF","+INF",1.0000,1.0000
"v2","+INF",1.0000,1.0000,"+INF","+INF","+INF","+INF"
"v3","+INF",1.0000,1.0000,"+INF","+INF","+INF","+INF"
"v4","+INF","+INF","+INF",1.0000,1.0000,1.0000,"+INF"
"v5","+INF","+INF","+INF","+INF",1.0000,"+INF","+INF"
"v6","+INF","+INF","+INF","+INF","+INF",1.0000,"+INF"
"v7",1.0000,1.0000,1.0000,"+INF","+INF",1.0000,1.0000

"MINRATIO","v1","v2","v3","v4","v5","v6","v7"
"v1",1.0000,0.0000,0.0000,0.0000,0.0000,0.0000,1.0000
"v2",1.0000,1.0000,1.0000,0.0000,0.0000,0.0000,1.0000
"v3",1.0000,1.0000,1.0000,0.0000,0.0000,0.0000,1.0000
"v4",0.0000,0.0000,0.0000,1.0000,0.0000,0.0000,0.0000
"v5",0.0000,0.0000,0.0000,1.0000,1.0000,0.0000,0.0000
"v6",1.0000,0.0000,0.0000,1.0000,0.0000,1.0000,1.0000
"v7",1.0000,0.0000,0.0000,0.0000,0.0000,0.0000,1.0000
```



Max V_1/V_6 = Inf & Min V_1/V_6 = 1 so they are Directionally Coupled (V6→ V1)

Max V_1/V_5 = Inf & Min V_1/V_5 = 0 so they are Uncoupled (V6→ V1)

Max V_3/V_2 = 1 & Min V_3/V_2 = 1 so they are Fully Coupled

Steps for Determining

1. Decouple reversible reactions into forward and reverse reactions, so $v_j \geq 0$
2. Remove reactions (k) which can not carry any flux, meaning given constraints $v_k = 0$.
3. Calculate the maximum and minimum flux ratios for pair-wise flux comparisons.

Distribution of Blocked Reactions

	H. pylori 389 rxns	*E. coli* 740 rxns	*S. cerevisiae* 1173 rxns
	number of blocked reactions		
Complex Media (Aerobic)	38	103	338
Glucose (Aerobic)	66	207	460
Glucose (Anaerobic)		210	515
Optimal Glucose (Aerobic)	77	408	774
Optimal Glucose (Anaerobic)		407	791

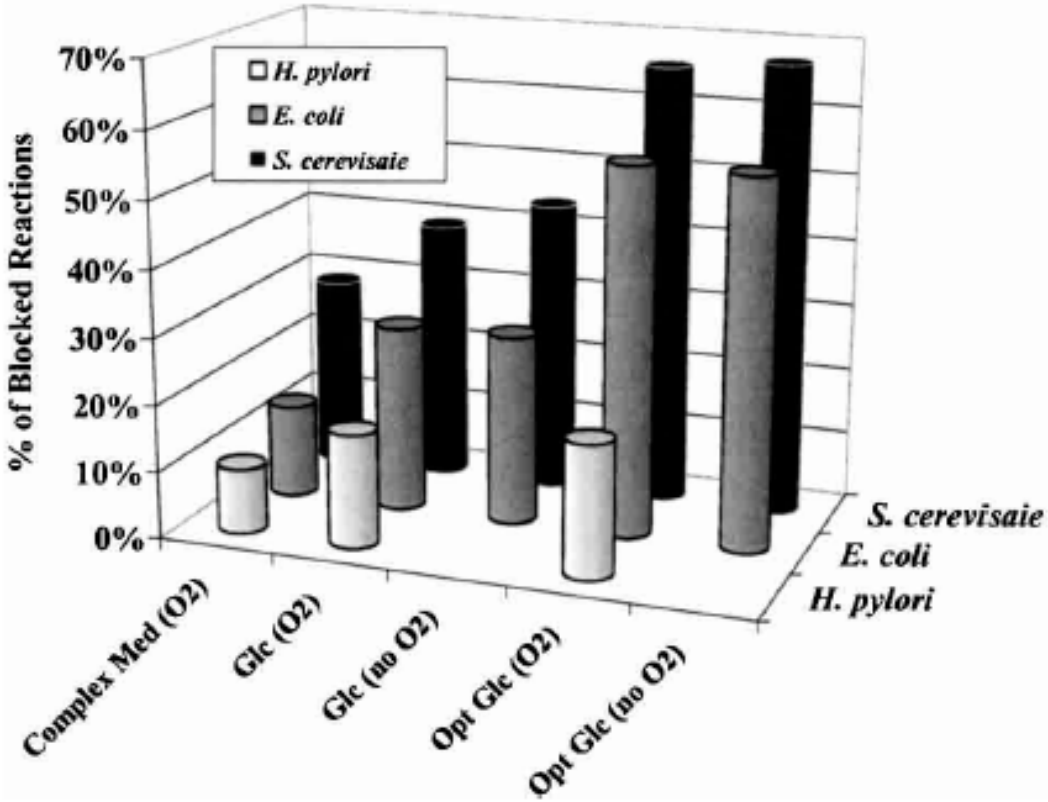


Figure 4 Total numbers and percentages of blocked reactions for the three networks under different growth conditions.

The number of blocked reactions (those which can not carry flux), depend on:

1) Network
2) Growth Condition

Burgard, AP, et al. Genome Research. 14(2):301-12 (2004).

Distribution of Coupled Reactions

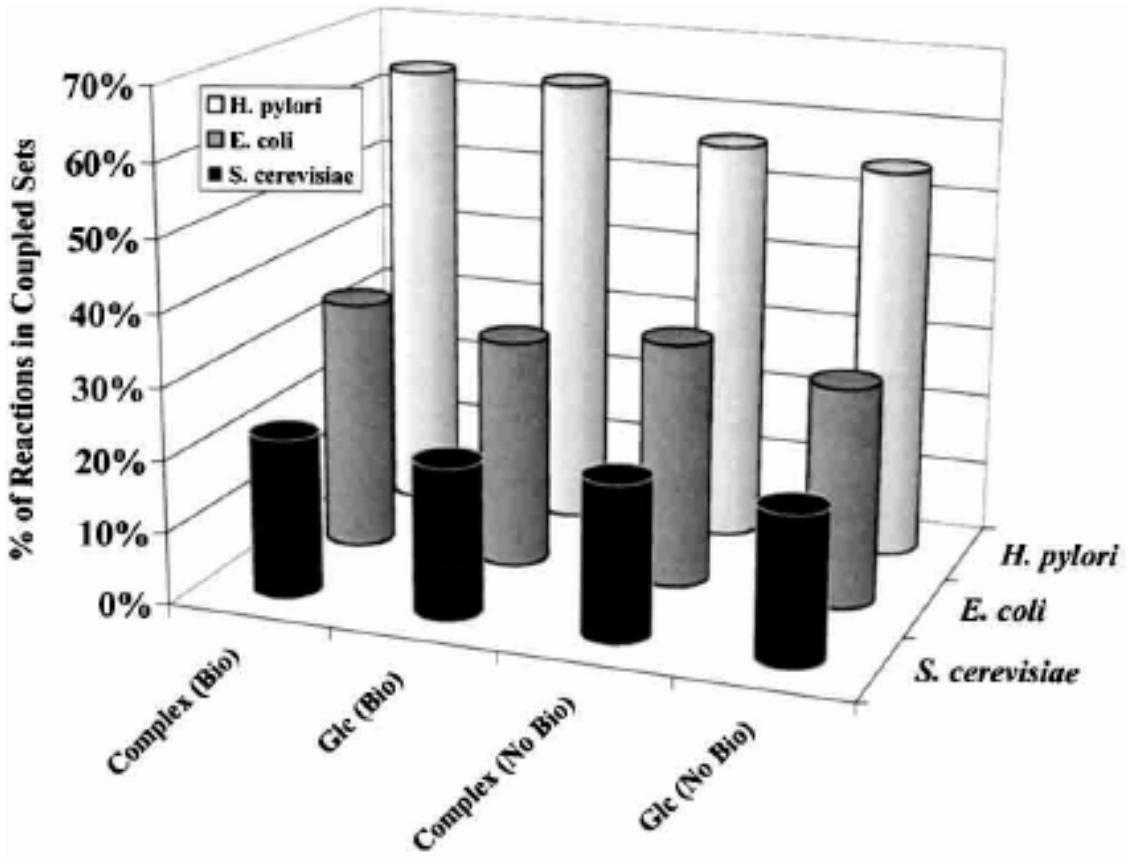


Figure 5 Percentage of reactions contained in coupled sets in the *H. pylori*, *E. coli*, and *S. cerevisiae* metabolic networks for growth on either a complex or glucose-minimal medium (with and without a biomass reaction).

Some Networks are More Highly Connected Leading Fewer Coupled Reactions:

1) Network
2) Growth Condition

Burgard, AP, et al. Genome Research. 14(2):301-12 (2004).

Large Coupled Reaction Sets

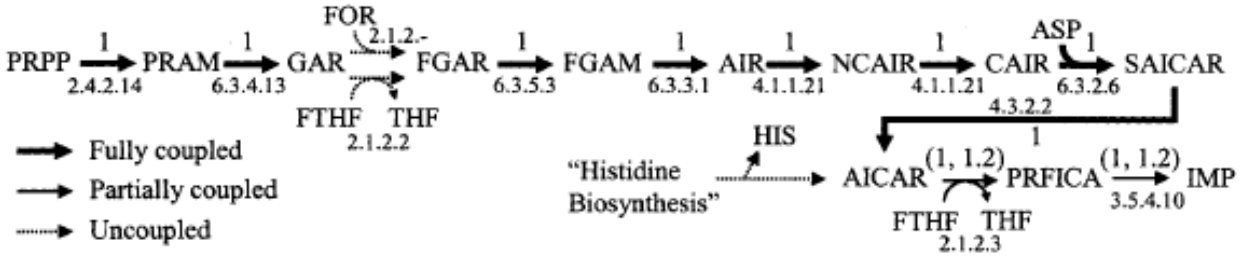


Figure 6 Coupled reaction set identified for purine biosynthesis in *E. coli* on a glucose-minimal medium, assuming a constant biomass composition. The numbers indicate the relative values or range of values for each flux in any particular flux distribution for given growth condition. Secondary metabolites and cofactors are omitted for simplicity.

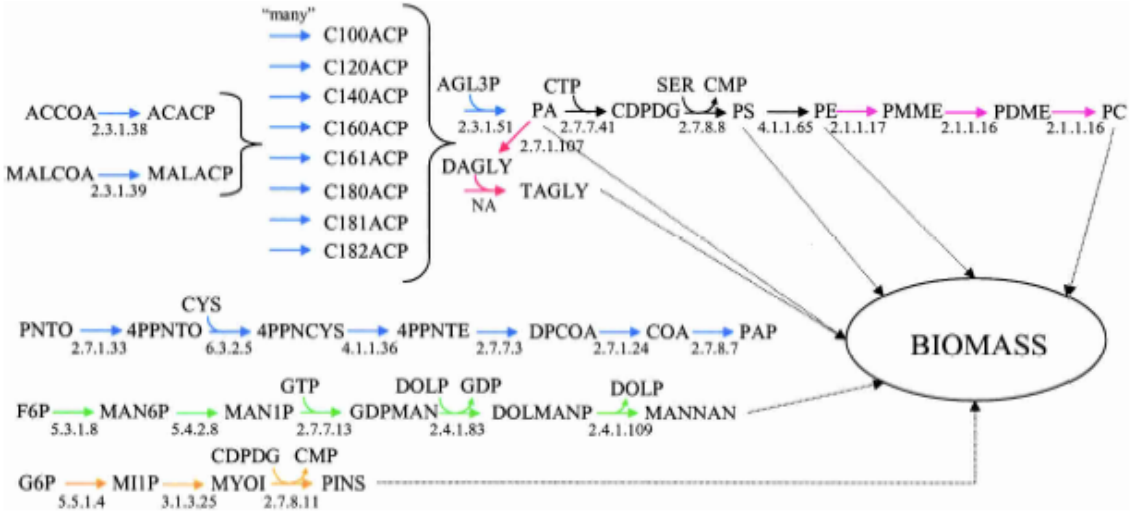


Figure 7 Reactions coupled to biomass formation for aerobic *S. cerevisiae* growth on a glucose-minimal medium. Secondary metabolites and cofactors are omitted for simplicity. All reactions are fully coupled, meaning that knowledge of one reaction flux is sufficient to specify the flux through all reactions at steady-state. Note that PAP is converted to AMP, which is a precursor to biomass. This enzyme subset is decomposed into numerous subsystems, indicated by different colored arrows, if the biomass reaction is replaced with drains on the various biomass precursors.

Burgard, AP, et al. Genome Research. 14(2):301-12 (2004).

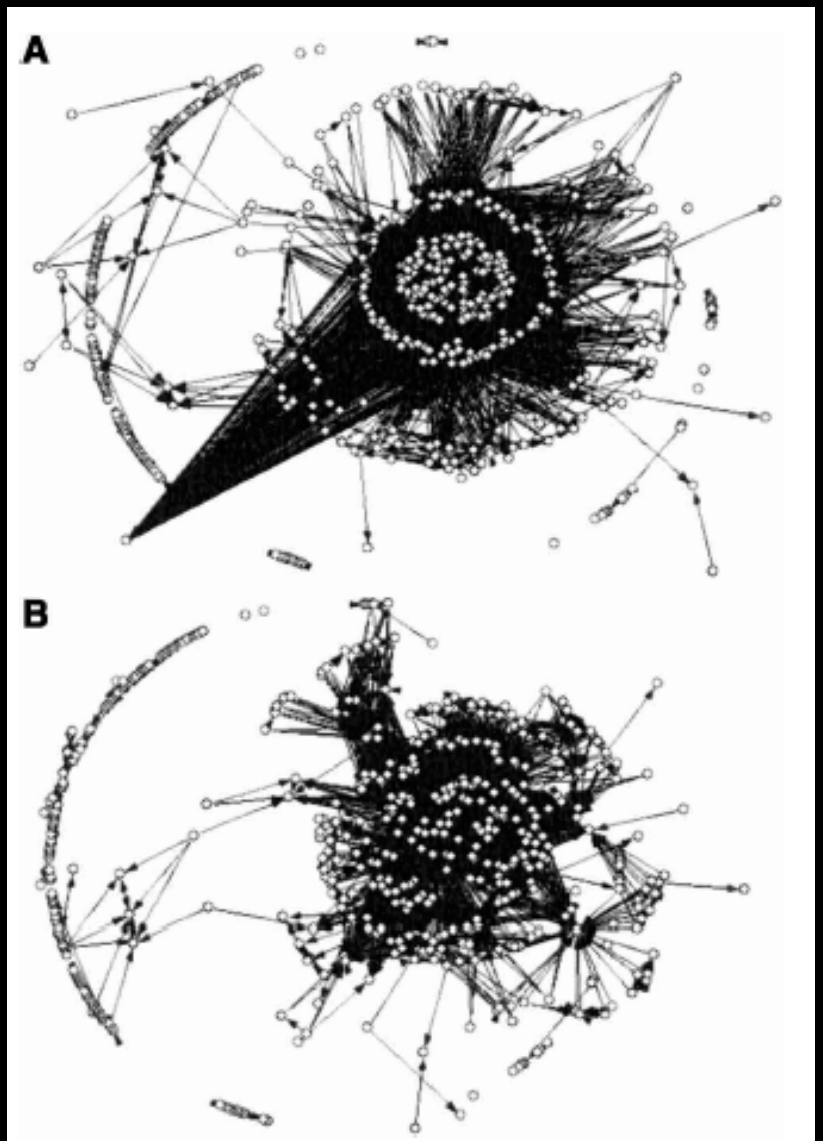


Figure 11 Genome-wide metabolic coupling for *E. coli* growth on a glucose-minimal medium with (*A*) or without (*B*) the presence of a biomass reaction. The biomass reaction is located in the *bottom left* corner of (*A*).

Inclusion of biomass reaction leads to a large number of coupled reactions

Burgard, AP, et al. Genome Research. 14(2):301-12 (2004).