Cellular Metabolism and Transport Processes

A. Introduction

Catabolism – degradation of a compound into smaller and simpler products with the concomitant generation of energy.

Anabolism – synthesis of more complex molecules for cellular processes with the utilization of energy.

Metabolism – catabolism and anabolism.

Currency of Biochemistry:

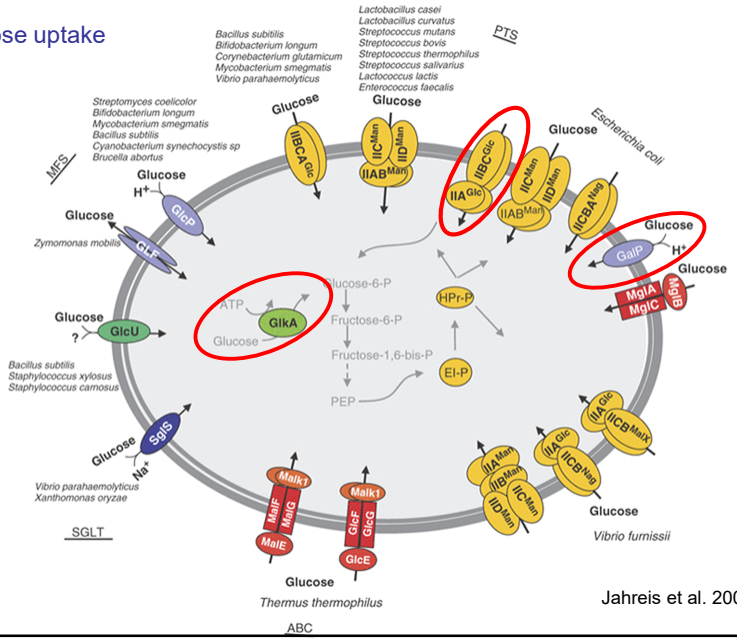
ATP – currency of energy
NADH – currency of "reducing power", electrons or protons
NADPH – currency of "reducing power"...for biomass synthesis

Websites for Enzymes/Biochemical Pathways:

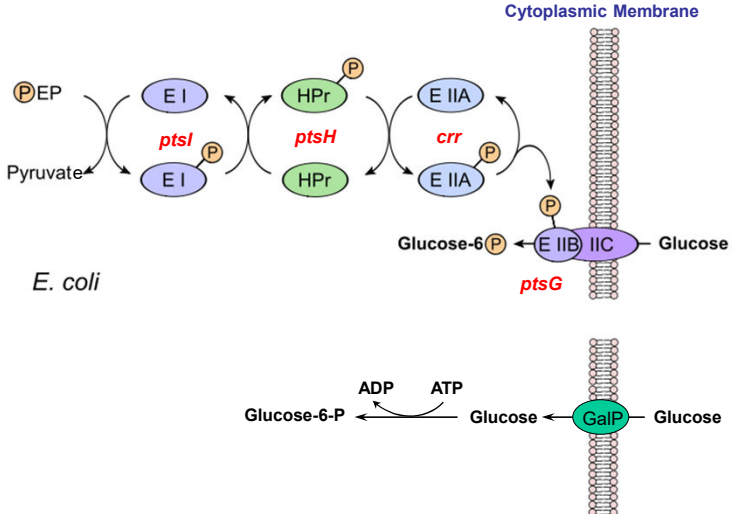
KEGG – http://www.genome.jp/kegg/pathway.html
BRENDA – http://www.brenda-enzymes.info/

B. Central Metabolic Pathways

1. Glucose uptake



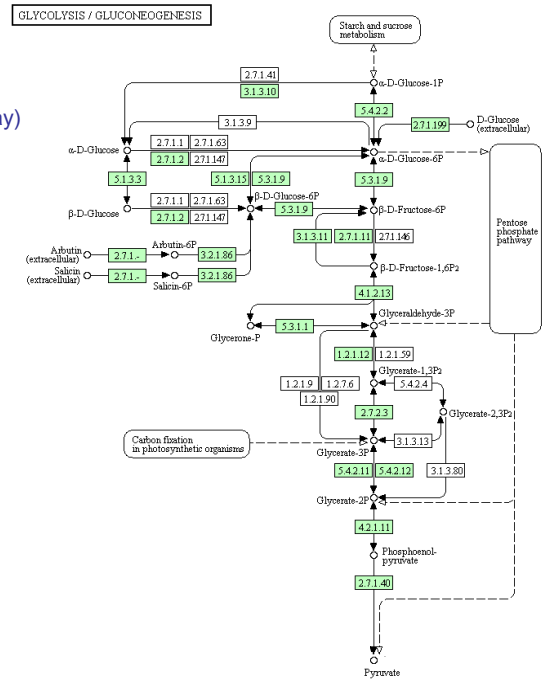
Jahreis et al. 2008



Complex regulation (for example):
glucose-6P binds to transcriptional activator SgrR forming SgrR*
SgrR* induces synthesis of SgrS RNA
SgrS RNA binds to RNA of chaperone Hfq
and ultimately, to ptsG RNA, which induces degradation of ptsG RNA by RNAase E.

2. Glycolysis

(Embden-Meyerhof-Parnas Pathway)



2. Embden-Meyerhof-Parnas Pathway (*E. coli* via PTS)

EC	gene	reaction
2.7.1.69	*ptsG* *malX* *crr*	glucose + ~~PEP~~ → ~~glucose-6P~~ + pyruvate
5.3.1.9	*pgi*	~~glucose-6P~~ → ~~fructose-6P~~
2.7.1.11	*pfkA* *pfkB*	~~fructose-6P~~ + ~~ATP~~ → ~~fructose-1,6P$_2$~~ + ~~ADP~~
4.1.2.13	*fbaA* *fbaB*	~~fructose-1,6P$_2$~~ → ~~glycerone-P~~ + ~~glyceraldehyde-3P~~
5.3.1.1	*tpiA*	~~glycerone-P~~ ←→ ~~glyceraldehyde-3P~~
1.2.1.12	*gapA*	~~2glyceraldehyde-3P~~ + 2Pi + 2NAD → ~~2(3P-glycerate-P)~~ + 2NADH
2.7.2.3	*pgk*	~~2(3P-glycerate-P)~~ + 2ADP → ~~2glycerate-3P~~ + 2ATP
5.4.2.11 5.4.2.12	*gpmA* *gpmM* *ytjC*	~~2glycerate-3P~~ ←→ ~~2glycerate-2P~~
4.2.1.11	*eno*	~~2glycerate-2P~~ → ~~2PEP~~ + 2H$_2$O
2.7.1.40	*pykA* *pykF*	~~PEP~~ + ~~ADP~~ → pyruvate + ~~ATP~~
Overall Reaction		**glucose + 2NAD + 2Pi + 2ADP → 2pyruvate + 2NADH + 2ATP + 2H$_2$O**

2. Embden-Meyerhof-Parnas Pathway (*E. coli* via glucokinase)

EC	gene	reaction
2.7.1.2	*glk*	glucose + ATP → glucose-6P + ADP
5.3.1.9	*pgi*	glucose-6P → fructose-6P
2.7.1.11	*pfkA* *pfkB*	fructose-6P + ATP → fructose-1,6P_2 + ADP
4.1.2.13	*fbaA* *fbaB*	fructose-1,6P_2 → glycerone-P + glyceraldehyde-3P
5.3.1.1	*tpiA*	glycerone-P ←→ glyceraldehyde-3P
1.2.1.12	*gapA*	2glyceraldehyde-3P + 2Pi + 2NAD → 2(3P-glycerate-P) + 2NADH
2.7.2.3	*pgk*	2(3P-glycerate-P) + 2ADP → 2glycerate-3P + 2ATP
5.4.2.11 5.4.2.12	*gpmA* *gpmM* *ytjC*	2glycerate-3P ←→ 2glycerate-2P
4.2.1.11	*eno*	2glycerate-2P → 2PEP + 2H_2O
2.7.1.40	*pykA* *pykF*	2PEP + 2ADP → 2pyruvate + 2ATP
Overall Reaction		**glucose + 2NAD + 2Pi + 2ADP → 2pyruvate + 2NADH + 2ATP + 2H_2O**

2. Embden-Meyerhof-Parnas Pathway (Key enzymes)

Enzyme	gene	reaction
phosphoglucose isomerase	*pgi*	glucose-6P → fructose-6P
phosphofructokinase	*pfkA* *pfkB*	fructose-6P + ATP → fructose-1,6P_2 + ADP
triose-phosphate isomerase	*tpiA*	glycerone-P ←→ glyceraldehyde-3P
glyceraldehyde-3P dehydrogenase	*gapA*	glyceraldehyde-3P + Pi + NAD → 3P-glycerate-P + NADH
phosphoglycerate kinase	*pgk*	3P-glycerate-P + ADP → glycerate-3P + ATP
phosphopyruvate hydratase "enolase"	*eno*	glycerate-2P → PEP + H_2O
pyruvate kinase	*pykA* *pykF*	PEP + ADP → pyruvate + ATP

2. Embden-Meyerhof-Parnas Pathway (Summary)

$$\text{glucose} + 2\text{ADP} + 2\text{NAD} + 2\text{Pi} \rightarrow 2\text{pyruvate} + 2\text{ATP} + 2\text{NADH} + 2\text{H}_2\text{O}$$

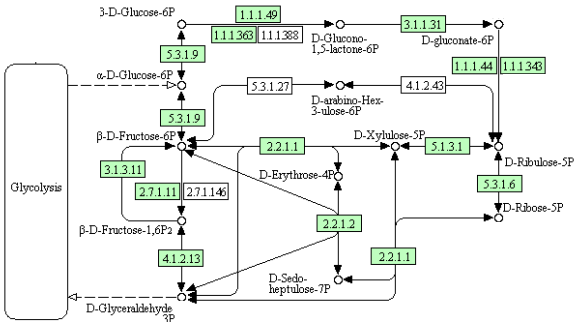
Can occur in the absence of oxygen if cells have mechanism to get rid of NADH and ATP generated.

Typically does not generate NADPH.

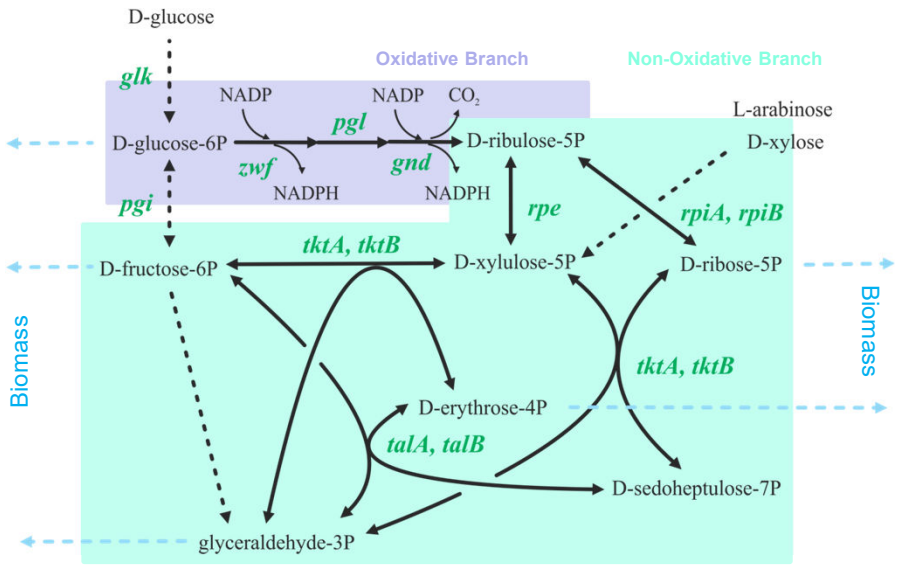
Goal is to break carbohydrates down into smaller components (i.e., "precursors" and "building blocks") found in "central metabolism".

Note subtle difference between glucose uptake via PTS compared to glucose uptake via glucokinase. PTS commits 50% of glucose to pyruvate! This fact makes a difference if product is derived from an intermediate "above" PEP.

3. Pentose phosphate pathway



3. Pentose phosphate pathway



3. Pentose phosphate pathway (*E. coli* via PTS)

EC	gene	reaction
2.7.1.69	*ptsG* *malX* *crr*	3glucose + 3PEP → 3glucose-6P + 3pyruvate
1.1.1.49	*zwf*	3glucose-6P + 3NADP → 3(6P-glucolactone) + 3NADPH
3.1.1.31	*pgl*	3(6P-glucolactone) + 3H_2O → 3gluconate-6P
1.1.1.44	*gnd*	3gluconate-6P + 3NADP → 3ribulose-5P + 3CO_2 + 3NADPH
5.1.3.1	*rpe*	2ribulose-5P ←→ 2xylulose-5P
5.3.1.6	*rpiA* *rpiB*	ribulose-5P ←→ ribose-5P
2.2.1.1	*tktA* *tktB*	xylulose-5P + ribose-5P ←→ sedoheptulose-7P + glyceraldehyde-3P
2.2.1.1	*tktA* *tktB*	xylulose-5P + erythrose-4P ←→ fructose-6P + glyceraldehyde-3P
2.2.1.2	*talA* *talB*	sedoheptulose-7P + glyceraldehyde-3P ←→ fructose-6P + erythrose-4P
Partial Reaction		**3glucose + 3PEP + 6NADP + 3H_2O → 3pyruvate + 6NADPH + 3CO_2 + 2fructose-6P + glyceraldehyde-3P**
Carbon Balance		3(6) + 3(3) → 3(3) + 3(1) + 2(6) + 1(3)

3. Pentose phosphate pathway (*E. coli* via lower EMP pathway)

EC	gene	reaction
Partial Reaction		**3glucose + 3PEP + 6NADP + $3H_2O$ → 3pyruvate + 6NADPH + $3CO_2$ + 2fructose-6P + glyceraldehyde-3P**
2.7.1.11	*pfkA* *pfkB*	2fructose-6P + 2ATP → 2fructose-1,$6P_2$ + 2ADP
4.1.2.13	*fbaA* *fbaB*	2fructose-1,$6P_2$ → 2glycerone-P + 2glyceraldehyde-3P
5.3.1.1	*tpiA*	2glycerone-P ←→ 2glyceraldehyde-3P
1.2.1.12	*gapA*	5glyceraldehyde-3P + 5Pi + 5NAD → 5(3P-glycerate-P) + 5NADH
2.7.2.3	*pgk*	5(3P-glycerate-P) + 5ADP → 5glycerate-3P + 5ATP
5.4.2.11 5.4.2.12	*gpmA* *gpmM* *ytjC*	5glycerate-3P ←→ 5glycerate-2P
4.2.1.11	*eno*	5glycerate-2P → 5PEP + $5H_2O$
2.7.1.40	*pykA* *pykF*	2PEP + 2ADP → 2pyruvate + 2ATP
Overall Reaction		**3glucose + 6NADP + 5NAD + 5Pi + 5ADP → 5pyruvate + $3CO_2$ + 6NADPH + 5NADH + 5ATP + $2H_2O$**

3. Pentose phosphate pathway (Key enzymes)

Enzyme	gene	reaction
glucose-6P dehydrogenase "zwischen ferment"	*zwf*	glucose-6P + NADP → 6P-glucolactone + NADPH
phosphogluconate dehydrogenase	*gnd*	gluconate-6P + NADP → ribulose-5P + CO_2 + NADPH
transketolase	*tktA* *tktB*	xylulose-5P + ribose-5P ←→ sedoheptulose-7P + glyceraldehyde-3P xylulose-5P + erythrose-4P ←→ fructose-6P + glyceraldehyde-3P
transaldolase	*talA* *talB*	sedoheptulose-7P + glyceraldehyde-3P ←→ fructose-6P + erythrose-4P

3. Pentose phosphate pathway (Summary)

3glucose + 6NADP + 5NAD + 5Pi + 5ADP →
5pyruvate + $3CO_2$ + 6NADPH + 5NADH + 5ATP + $2H_2O$

Can occur in the absence of oxygen if cells have mechanism to get rid of NADPH & NADH generated.

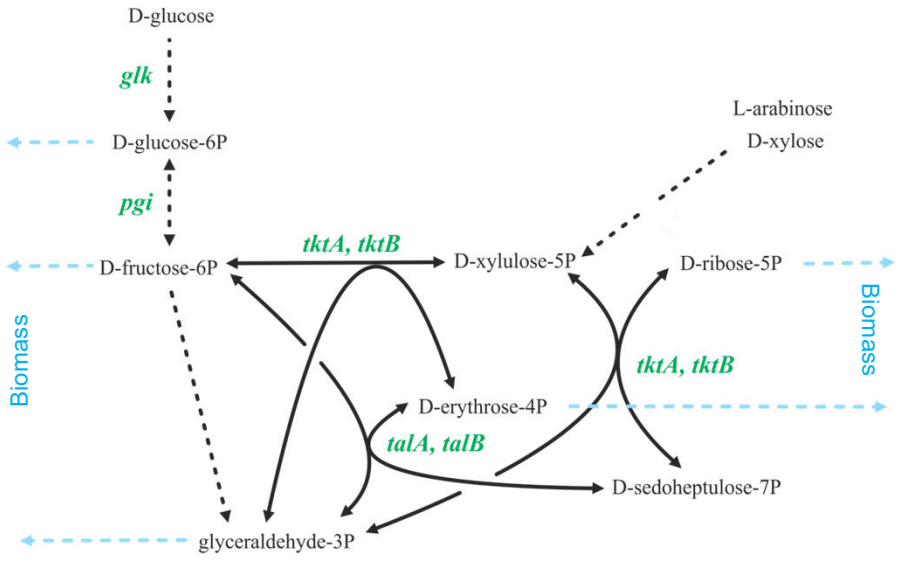
Goals are to generate NADPH and 5-carbon and 4-carbon compounds used for the synthesis of biomass.

Note that, like we observed for EMP pathway, glucose uptake via PTS generates same overall result as glucose uptake via glucokinase if the end product is pyruvate (!)

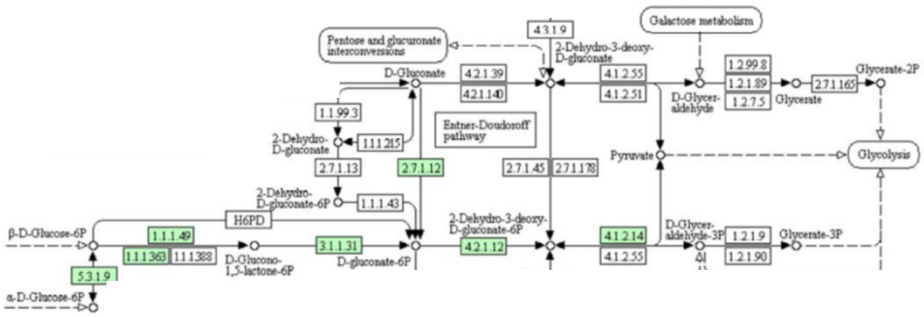
Because transaldolase and transketolase are reversible, knocking out entry into PP pathway (e.g., *zwf*) still allows formation of biomass precursor molecules erythrose-4P and ribose-5P.

Note that despite losing a carbon from glucose-6P as CO_2, 66% of the carbon returns to fructose-6P.

3. Pentose phosphate pathway (Removing oxidative path)



4. Entner-Doudoroff pathway (*E. coli* via PTS)



Enzyme	gene	reaction
phoshogluconate dehydratase (EC 4.2.1.12)	*edd*	gluconate-6P → 2-dehydro-3-deoxy-gluconate-6P + H_2O
2-dehydro-3-deoxyphosphogluconate aldolase (EC 4.1.2.14)	*eda*	2-dehydro-3-deoxy-gluconate-6P → pyruvate + glyceraldehyde-3P

4. Entner-Doudoroff pathway (*E. coli* via PTS)

EC	gene	reaction
2.7.1.69	*ptsG* *malX* *crr*	glucose + PEP → glucose-6P + pyruvate
1.1.1.49	*zwf*	glucose-6P + NADP → 6P-glucolactone + NADPH
3.1.1.31	*pgl*	6P-glucolactone + H_2O → gluconate-6P
4.2.1.12	*edd*	gluconate-6P → 2-dehydro-3-deoxy-gluconate-6P + H_2O
4.1.2.14	*eda*	2-dehydro-3-deoxy-gluconate-6P → pyruvate + glyceraldehyde-3P
1.2.1.12	*gapA*	glyceraldehyde-3P + Pi + NAD → 3P-glycerate-P + NADH
2.7.2.3	*pgk*	3P-glycerate-P + ADP → glycerate-3P + ATP
5.4.2.11 5.4.2.12	*gpmA* *gpmM* *ytjC*	glycerate-3P ←→ glycerate-2P
4.2.1.11	*eno*	glycerate-2P → PEP + H_2O
Overall Reaction		**glucose + NADP + NAD + ADP + Pi → 2pyruvate + NADPH + NADH + ATP + H_2O**

Comparison of EMP pathway and pentose phosphate pathway

EMP Pathway

glucose + 2NAD + 2Pi + 2ADP →
2pyruvate + 2NADH + 2ATP + $2H_2O$

Pentose Phosphate Pathway

3glucose + 6NADP + 5NAD + 5Pi + 5ADP →
5pyruvate + $3CO_2$ + 6NADPH + 5NADH + 5ATP + $2H_2O$

Entner-Doudoroff Pathway

glucose + NADP + NAD + ADP + Pi →
2pyruvate + NADPH + NADH + ATP + H2O

Comparison of glucose consumption pathways

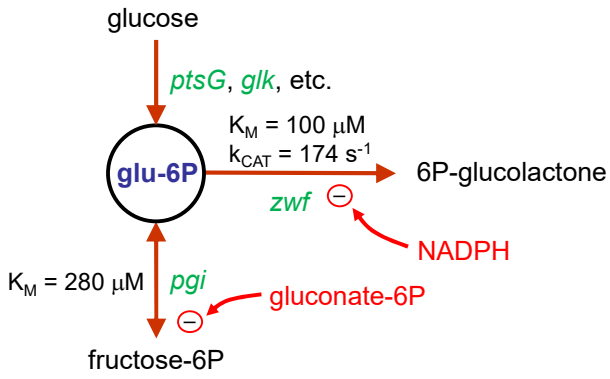
Parameter	EMP Pathway	PP Pathway	ED Pathway
$Y_{ATP/GLU}$	2.00 mol/mol	1.67 mol/mol	1.00 mol/mol
$Y_{NADH/GLU}$	2.00 mol/mol	1.67 mol/mol	1.00 mol/mol
$Y_{NADPH/GLU}$	0.00 mol/mol	2.00 mol/mol	1.00 mol/mol
$Y_{PYR/GLU}$	2.00 mol/mol	1.67 mol/mol	2.00 mol/mol
$Y_{CO2/GLU}$	0.00 mol/mol	1.00 mol/mol	0.00 mol/mol

Because the Entner-Doudoroff Pathway generates so little ATP, organisms often must have a high rate of glucose uptake to generate enough ATP for biomass, and generate a by-product *(Zymomonas mobilis*). Thus, the biomass yield on glucose is very low.

The Pentose Phosphate Pathway, which immediately oxidizes a portion of the carbohydrate substrate to CO_2, also generates the most electrons and reduced cofactors (3.67 mol/mol total NADH + NADPH).

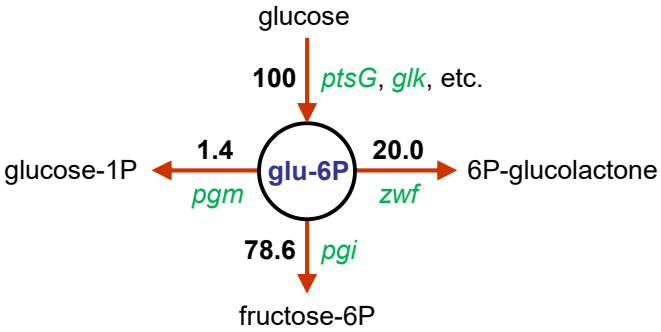
Comparison of EMP pathway and pentose phosphate pathway

Comparison of enzymes:



Comparison of EMP pathway and pentose phosphate pathway

For *E. coli* at growth rate of 0.2 h^{-1} on glucose:



20% of glucose taken up by cells enters the pentose phosphate pathway. Note this number is different at different growth rates, when additional gene knockouts are present, or under different growth conditions.

Nicolas et al. 2007
Zhao et al. 2004

Comparison of EMP pathway and pentose phosphate pathway

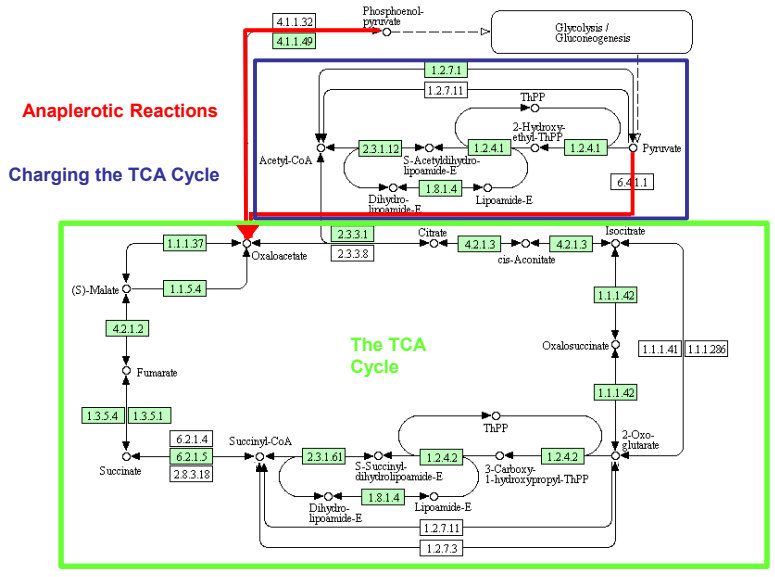
Based on 20.0% glucose entering the pentose phosphate pathway at a growth rate of 0.2 h^{-1}, the true stoichiometric equation to pyruvate *considering biomass formation* is:

100 glucose + 40.0 NADP + 174.0 NAD + 174.0 Pi + 108.7 ADP → 20.0 CO_2 + 135.9 pyruvate + 40.0 NADPH + 174.0 NADH + 108.7 ATP + 139.0 H_2O + biomass

biomass = 1.4 glucose-6P + 6.2 ribose-5P + 2.7 erythrose-4P
+ 0.7 fructose-6P + 1.2 glyceraldehyde-3P
+ 15.0 phosphoglycerate + 3.1 PEP + (18.9 pyruvate
+ 25.9 acetyl-CoA + 7.5 2-oxoglutarate + 12.5 oxaloacetate)

Parameter	EMP Pathway	Pentose Phosphate Pathway	Reality
$Y_{ATP/GLU}$	2.00 mol/mol	1.67 mol/mol	1.09 mol/mol
$Y_{NADH/GLU}$	2.00 mol/mol	1.67 mol/mol	1.74 mol/mol
$Y_{NADPH/GLU}$	0.00 mol/mol	2.00 mol/mol	0.40 mol/mol
$Y_{PYR/GLU}$	2.00 mol/mol	1.67 mol/mol	1.36 mol/mol
$Y_{CO2/GLU}$	0.00 mol/mol	1.00 mol/mol	0.20 mol/mol

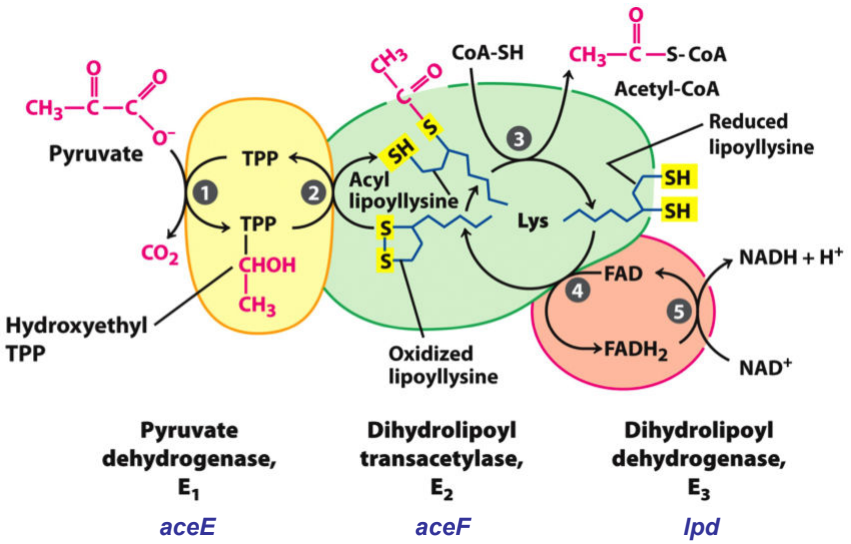
4. Tricarboxylic acid cycle



a. Charging the TCA cycle – The pyruvate dehydrogenase complex

EC	gene	reaction
1.2.4.1	*aceE*	pyruvate + thiamine pyrophosphate (TPP)-E1 → CO_2 + 2-hydroxyethyl-TPP-E1 2-hydroxyethyl-TPP-E1 + (lipoyl)lysine-E2 → TPP-E1 + (S-acetyldihydrolipoyl)lysine-E2
2.3.1.12	*aceF*	(S-acetyldihydrolipoyl)lysine-E2 + HS-CoA → (dihydrolipoyl)lysine-E2 + acetyl-CoA
1.8.1.4	*lpd*	(dihydrolipoyl)lysine-E2 + FAD-E3 → (lipoyl)lysine-E2 + FADH-E3 FADH-E3 + NAD → FAD-E3 + NADH
Overall Reaction		**pyruvate + NAD + HS-CoA → acetyl-CoA + CO_2 + NADH**

a. Charging the TCA cycle – The pyruvate dehydrogenase complex



b. The TCA cycle

EC	gene	reaction
2.3.3.1	*gltA*	acetyl-CoA + H_2O + oxaloacetate → citrate + HS-CoA
4.2.1.3	*acnB*	citrate → cis-aconitate + H_2O cis-aconitate + H_2O → isocitrate
1.1.1.42	*icd*	isocitrate + NADP → oxalosuccinate + NADPH oxalosuccinate → 2-oxoglutarate + CO_2
1.2.4.2	*sucA*	2-oxoglutarate + TPP-E1 → CO_2 + HOOC-CH_2-CH_2-C(OH)-TPP-E1 HOOC-CH_2-CH_2-C(OH)-TPP-E1 + (lipoyl)lysine-E2 → TPP-E1 + (S-succinyldihydrolipoyl)lysine-E2
2.3.1.61	*sucB*	(S-succinyldihydrolipoyl)lysine-E2 + HS-CoA → (dihydrolipoyl)lysine-E2 + succinyl-CoA
1.8.1.4	*lpd*	(dihydrolipoyl)lysine-E2 + FAD-E3 → (lipoyl)lysine-E2 + FADH-E3 FADH-E3 + NAD → FAD-E3 + NADH
6.2.1.5	*sucC*	succinyl-CoA + ADP + Pi → succinate + HS-CoA + ATP
1.3.5.1	*sdhA* *sdhB* *sdhC* *sdhD*	succinate + ubiquinone (membrane) → fumarate + ubiquinol

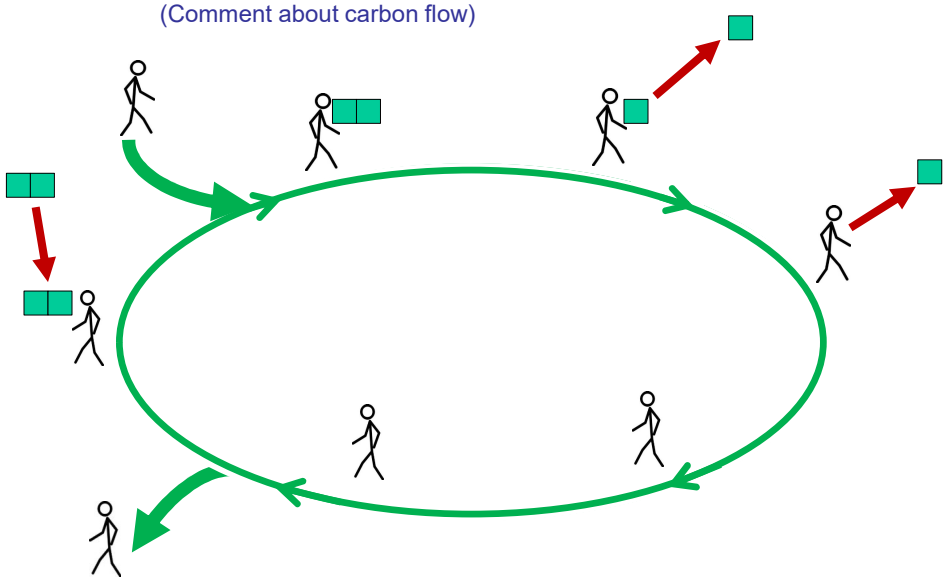
b. The TCA cycle

EC	gene	reaction
4.2.1.2	*fumC*	fumarate + H_2O → malate
1.1.1.37	*mdh*	malate + NAD → oxaloacetate + NADH
Overall Reaction		**acetyl-CoA + $2H_2O$ + NADP + 2NAD + ADP + Pi + ubiquinone → $2CO_2$ + NADPH + 2NADH + ATP + ubiquinol**

b. The TCA cycle
(Key enzymes)

Enzyme	gene	reaction
citrate synthase	*gltA*	acetyl-CoA + H_2O + oxaloacetate → citrate + HS-CoA
isocitrate dehydrogenase	*icd*	isocitrate + NADP → oxalosuccinate + NADPH oxalosuccinate → 2-oxoglutarate + CO_2
malate dehydrogenase	*mdh*	malate + NAD → oxaloacetate + NADH

b. The TCA cycle
(Comment about carbon flow)



c. Anaplerotic pathways (replenishing the TCA cycle)

Goal: Form TCA cycle intermediates from metabolites outside TCA cycle

i. PEP carboxylase (*ppc*)
ii. PEP carboxykinase (*pckA*)
iii. pyruvate carboxylase (*pyc*, not found in *E. coli*)

EC	k_{CAT}	gene	reaction
4.1.1.31	540 s^{-1}	*ppc*	PEP + HCO_3^- → oxaloacetate + Pi
4.1.1.49	0.67 s^{-1}	*pckA*	PEP + CO_2 + ADP → oxaloacetate + ATP*
6.4.1.1		*pyc*	pyruvate + HCO_3^- + ATP → oxaloacetate + ADP + Pi

*Often operates in reverse direction:
oxaloacetate + ATP → PEP + CO_2 + ADP

c. Anaplerotic pathways (replenishing the TCA cycle)

iv. Glyoxylate Shunt

EC	gene	reaction
2.3.3.9	*glcB*	acetyl CoA + glyoxylate + H_2O → malate + HS-CoA
4.1.3.1	*aceA*	isocitrate → succinate + glyoxylate
2.3.3.1	*gltA*	acetyl-CoA + H_2O + oxaloacetate → citrate + HS-CoA
4.2.1.3	*acnB*	citrate → cis-aconitate + H_2O cis-aconitate + H_2O → isocitrate
1.3.5.1	*sdhA* *sdhB* *sdhC* *sdhD*	succinate + ubiquinone (membrane) → fumarate + ubiquinol
4.2.1.2	*fumC*	fumarate + H_2O → malate
1.1.1.37	*mdh*	2malate + 2NAD → 2oxaloacetate + 2NADH
Overall Reaction		**2acetyl-CoA + 3H_2O + 2NAD + ubiquinone → oxaloacetate + 2NADH + ubiquinol**

c. Anaplerotic pathways
(Key enzymes)

Enzyme	gene	reaction
PEP carboxylase	*ppc*	PEP + HCO_3^- → oxaloacetate + Pi
PEP carboxykinase	*pckA*	PEP + CO_2 + ADP → oxaloacetate + ATP*
pyruvate carboxylase	*pyc*	pyruvate + HCO_3^- + ATP → oxaloacetate + ADP + Pi
malate synthase	*glcB*	acetyl CoA + glyoxylate + H_2O → malate + HS-CoA
isocitrate lyase	*aceA*	isocitrate → succinate + glyoxylate

Comparison of TCA cycle and glyoxylate shunt

TCA Cycle

acetyl-CoA + $2H_2O$ + NADP + 2NAD + ADP + Pi + ubiquinone →
$2CO_2$ + NADPH + 2NADH + ATP + ubiquinol

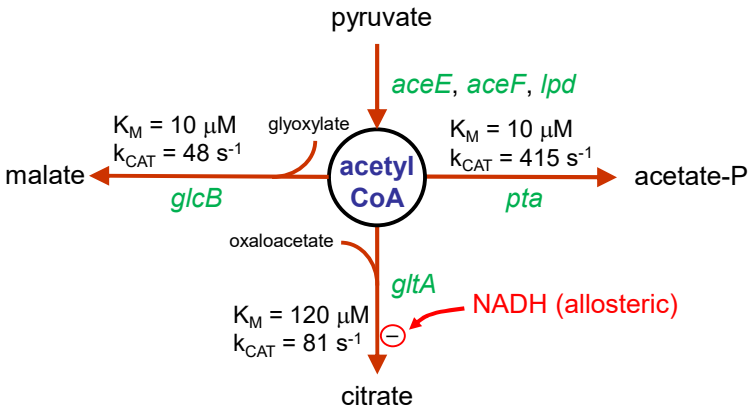
Glyoxylate Shunt

2acetyl-CoA + $3H_2O$ + 2NAD + ubiquinone →
oxaloacetate + 2NADH + ubiquinol

Parameter	TCA Cycle	Glyoxylate Shunt
$Y_{ATP/AcCoA}$	1.00 mol/mol	0.00 mol/mol
$Y_{NAD(P)H+UbiH/AcCoA}$	4.00 mol/mol	1.50 mol/mol
$Y_{OAA/AcCoA}$	0.00 mol/mol	0.50 mol/mol
$Y_{CO2/AcCoA}$	2.00 mol/mol	0.00 mol/mol

Comparison of TCA cycle and glyoxylate shunt

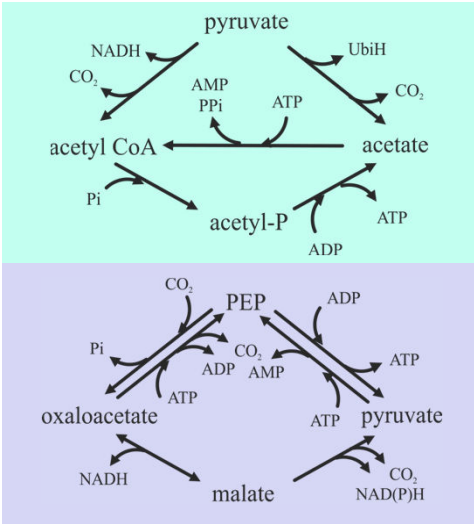
Comparison of enzymes:



5. Other important pathways (energy & electron dissipating)

EC	gene	reaction
1.2.5.1	*poxB*	pyruvate + H_2O + ubiquinone → acetate + ubiquinol + CO_2
2.3.1.8 2.7.2.1	*pta* *ackA*	acetyl-CoA + Pi → HS-CoA + acetyl-P acetyl-P + ADP → acetate + ATP
6.2.1.1	*acs*	acetate + HS-CoA + ATP → acetyl-CoA + AMP + PPi
1.6.1.1	*udhA* *sthA*	NADP + NADH ←→ NADPH + NAD
4.1.1.31 4.1.1.49	*ppc* *pckA*	PEP + HCO_3^- → oxaloacetate + Pi oxaloacetate + ATP → PEP + CO_2 + ADP
1.1.1.38 1.1.1.40	*sfcA* *maeB*	malate + NAD → pyruvate + CO_2 + NADH malate + NADP → pyruvate + CO_2 + NADPH
2.7.9.2	*pykA* *ppsA*	PEP + ADP → pyruvate + ATP pyruvate + ATP + H_2O → PEP + AMP + Pi

5. Other important pathways (energy & electron dissipating)



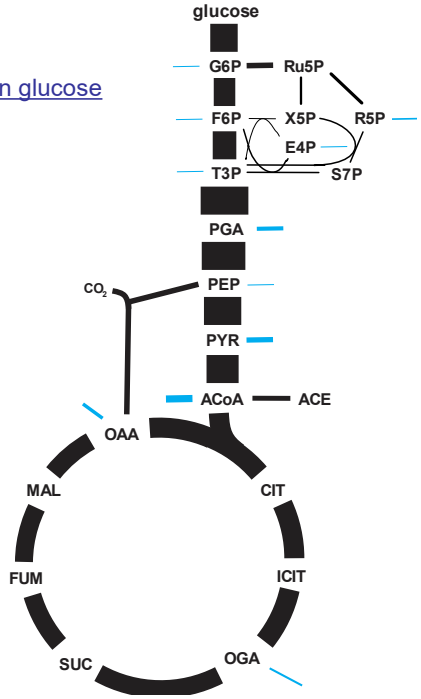
5. Other important pathways (Key enzymes)

Enzyme	gene	reaction
pyruvate oxidase	*poxB*	pyruvate + H_2O + ubiquinone → acetate + ubiquinol + CO_2
phosphotransacetylase	*pta*	acetyl-CoA + Pi → HS-CoA + acetyl-P
acetate kinase	*ackA*	acetyl-P + ADP → acetate + ATP
acetyl CoA synthase	*acs*	acetate + HS-CoA + ATP → acetyl-CoA + AMP + PPi
transhydrogenase	*udhA* *sthA*	NADP + NADH ←→ NADPH + NAD
"malic enzymes"	*sfcA* *maeB*	malate + NAD → pyruvate + CO_2 + NADH malate + NADP → pyruvate + CO_2 + NADPH
PEP synthase	*ppsA*	pyruvate + ATP + H_2O → PEP + AMP + Pi

True fluxes (*E. coli* growing aerobically on glucose at a growth rate of 0.2 h^{-1})

Comments

- PEP carboxylase is principal anaplerotic reaction. The difference of PEP carboxylase and PEP carboxykinase exactly balances withdrawal from TCA cycle intermediates oxaloacetate and 2-oxoglutarate.
- Not a lot of carbon flux travels through PP pathway.
- The largest withdrawal of carbon for biomass is acetyl CoA, then pyruvate.
- Essentially no carbon flux proceeds through glyoxylate shunt for wild-type *E. coli* growing on glucose.



Zhao et al. 2004

True fluxes (*E. coli* growing aerobically on glucose at a growth rate of 0.2 h^{-1})

Based on the true fluxes, one can calculate the overall stoichiometry of catabolism:

100 glucose + 113.1 NADP + 422.2 NAD + 65.6 ubiquinone + 61.4 Pi + 190.1 ADP → 255.7 CO_2 + 113.1 NADPH + 422.2 NADH + 65.6 ubiquinol + 190.1 ATP + 0.3 H_2O + 18.0 acetate + biomass

biomass = 1.4 glucose-6P + 6.2 ribose-5P + 2.7 erythrose-4P
+ 0.7 fructose-6P + 1.2 glyceraldehyde-3P
+ 15.0 phosphoglycerate + 3.1 PEP + 18.9 pyruvate
+ 25.9 acetyl-CoA + 7.5 2-oxoglutarate + 12.5 oxaloacetate

Parameter	Reality
$Y_{ATP/GLU}$	1.90 mol/mol
$Y_{NADH/GLU}$	4.22 mol/mol
$Y_{NADPH/GLU}$	1.13 mol/mol
$Y_{CO2/GLU}$	2.56 mol/mol

Zhao et al. 2004

True fluxes (*E. coli* growing aerobically on glucose at a growth rate of 0.2 h^{-1})

Pathway	ATP	NADH	NADPH	UbiH
EMP/PP	108.7	174.0	40.0	0
Charging	0	117.0	0	0
Anaplerotic	-2.2	0	0	0
TCA Cycle	65.6	131.2	73.1	65.6
Extra (acetate)	18.0	0	0	0
Total	**190.1**	**422.2**	**113.1**	**65.6**

57%

41%

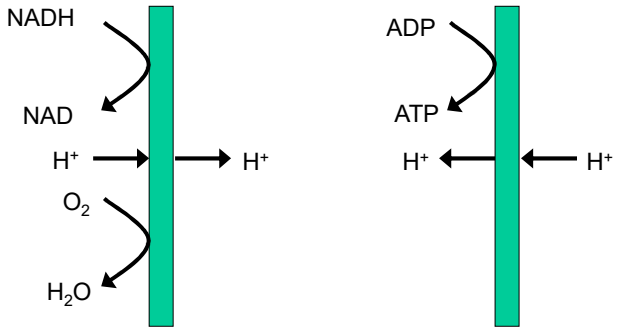
64%

Zhao et al. 2004

6. Balancing the electrons generated from oxidation of organic substrates

a. In the presence of O_2 – oxidative phosphorylation

This is a membrane bound system of reactions in which electrons are shuttled between chemical carriers. The result is:



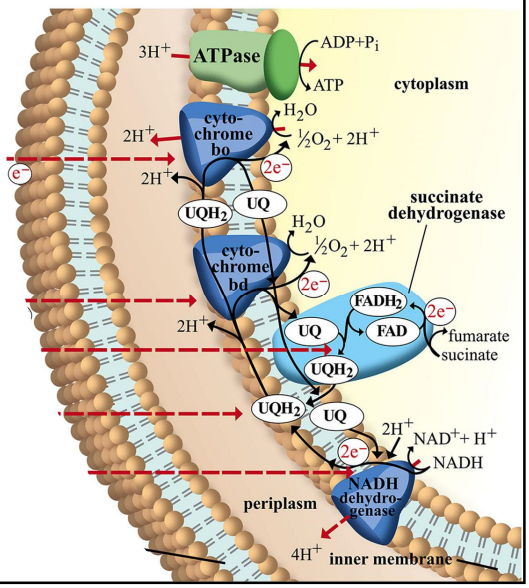
a. In the presence of O_2 – oxidative phosphorylation

E. coli

ATP is generated when H^+ reenters membrane to balance the concentration gradient. Usually generate 2-3 ATP per NADH

The amount of ATP generated from electrons (NADH and other reduced species) depends on environmental conditions, providing cell with metabolic flexibility:

- Na+/H+ antiporters
- flagella
- other pumps



Kracke et al. 2015

b. In the absence of O_2 – anaerobic metabolism

Cells have two general approaches to "get rid of" electrons generated in biochemical pathways:

i. *anaerobic respiration* – an electron acceptor different from O_2 is used.

$NO_3^- \rightarrow NO_2^-$ $\quad\quad$ $N_2O \rightarrow N_2$

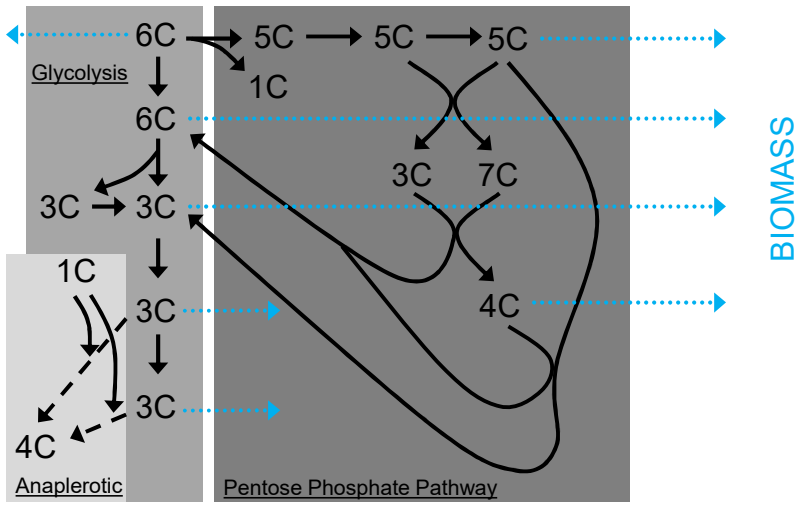
ii. *fermentation* – regeneration of NAD is accomplished by conversion of a chemical into a dead-end product.

pyruvate + NADH → lactate + NAD

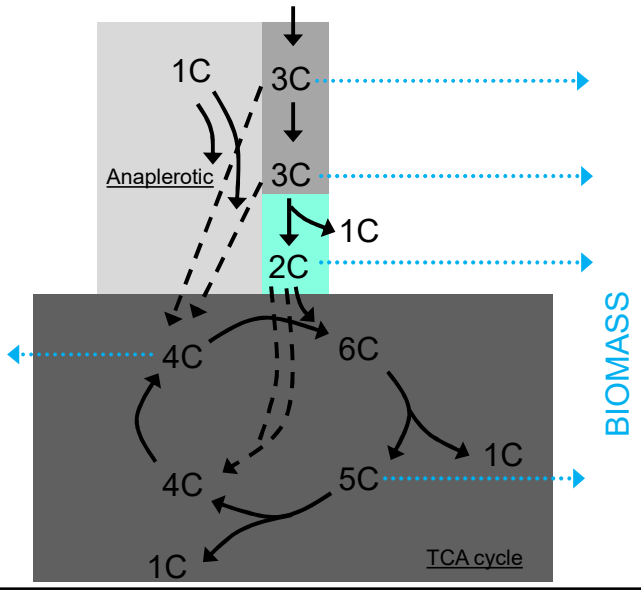
pyruvate + NADH → ethanol + CO_2 + NAD

Under anaerobic conditions, the electrons generated by oxidation of organic compounds for the generation of energy must be balanced exactly by the electrons consumed for the formation of biomass and for the production of by-products (e.g., via fermentation).

7. Summary of principal carbon flow (part 1)

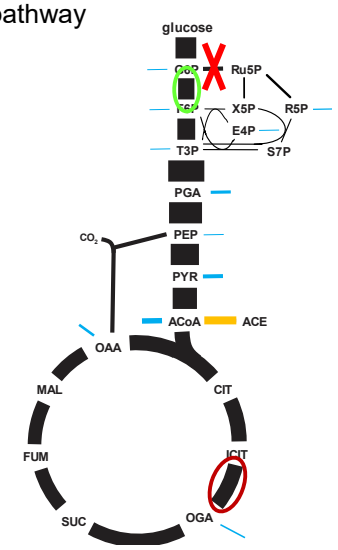


7. Summary of principal carbon flow (part 2)



C. Effect of Knockouts

1. *zwf* – glucose-6P 1-dehydrogenase (EC 1.1.1.49)
   This knockout blocks entry into PP pathway



<u>under batch growth:</u>
- small decrease in growth rate (<5%).

<u>at growth rate of 0.20 h^{-1}:</u>
- 49% increase in PGI activity.
- 35% increase in ICDH activity.
- 35% increase in CO_2 evolution (!)
- 91% increase in acetate formation.
- Increase in transhydrogenase activity.

Nicolas et al. 2007
Zhao et al. 2004

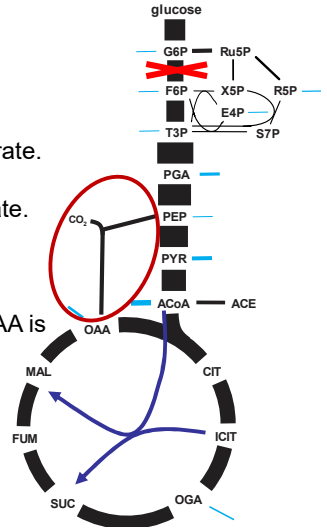
1. *zwf* – glucose-6P 1-dehydrogenase (EC 1.1.1.49)
   block entry into PP pathway

Glucose uptake = 100
Growth rate of 0.10 h^{-1}

	wild-type				Δ*zwf*			
Pathway	**ATP**	**NADH**	**NADPH**	**UbiH**	**ATP**	**NADH**	**NADPH**	**UbiH**
EMP/PP	107	175	58	0	122	184	0	0
Charging	0	109	0	0	0	114	0	0
Anaplerotic	-66	0	7	0	-47	0	7	0
TCA Cycle	80	152	89	80	83	159	93	83
Acetate	0	0	0	0	0	0	0	0
Transhydrogenase	0	16	-16	0	0	-46	46	0
Total	**121**	**452**	**138**	**80**	**158**	**411**	**146**	**83**

Hua et al. 2003

2. *pgi* – phosphoglucose isomerase (EC 5.3.1.9)
block entry into EMP pathway



under batch growth:
- 78% decrease in growth rate.
- 55% decrease in glucose consumption rate.
- Overexpression of *udhA* (transhydrogenase) increased growth rate.

at growth rate of 0.10 h^{-1}:
- decrease in PPC flux.
- induction of glyoxylate shunt. 50% of OAA is derived from glyoxylate shunt.

Hua et al. 2003
Canonaco et al. 2001

2. *pgi* – phosphoglucose isomerase (EC 5.3.1.9)
block entry into EMP pathway

Glucose uptake = 100
Growth rate of 0.10 h^{-1}

	wild-type				Δ*pgi*			
Pathway	**ATP**	**NADH**	**NADPH**	**UbiH**	**ATP**	**NADH**	**NADPH**	**UbiH**
EMP/PP	107	175	58	0	122	148	198	0
Charging	0	109	0	0	0	122	0	0
Anaplerotic	-66	0	7	0	-50	0	0	0
TCA Cycle	80	152	89	80	16	107	26	54
Acetate	0	0	0	0	0	0	0	0
Transhydrogenase	0	16	-16	0	0	72	-72	0
Total	**121**	**452**	**138**	**80**	**88**	**449**	**152**	**54**

Hua et al. 2003

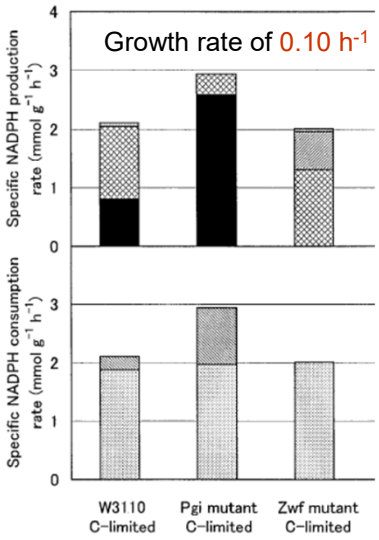
2. *pgi* – phosphoglucose isomerase (EC 5.3.1.9)
block entry into EMP pathway

How is NADPH formed?

- PP pathway
- TCA cycle (ICDH)
- Transhydrogenase (NADH + NADP → NAD + NADPH)
- Malic Enzyme

How is NADPH consumed?

- Biomass
- Transhydrogenase (NAD + NADPH → NADH + NADP)



Hua et al. 2003

3. *pykF* – pyruvate kinase (EC 2.7.1.40)
blocks conversion of PEP into pyruvate.
pykA is not active.

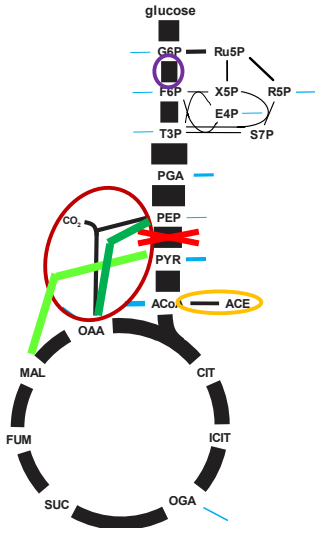
under batch growth:

- small decrease in growth rate (<5%).
- 66% decrease in acetate formation.
- 3× increase in malic enzyme activity.
- 2× decrease in citrate synthase activity.

at growth rate of 0.10 h^{-1}:

- >2.5× increase in PPC flux.
- Large increase in PCK and ME flux.
- 2× increase in flux through PP pathway.
- 60% decrease in PGI flux.
- almost an elimination of acetate formation.

Note: Pyruvate is still formed as a result of PTS-mediated glucose uptake!

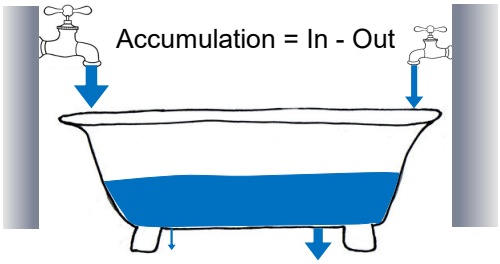


Al Said Siddiquee et al. 2004

D. Material Balances

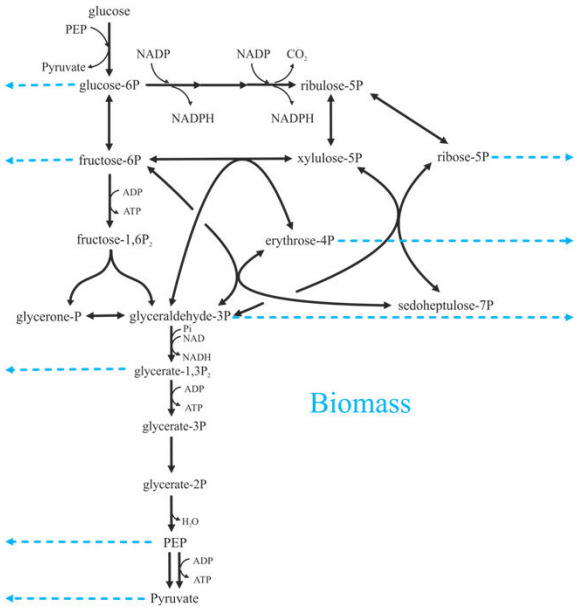
The amount of "flux" (mmol/Lh) through each pathway can be calculated by knowing the rates of carbon source consumption, product formation, and the composition of the cell. As an illustration, we'll consider metabolism of glucose to pyruvate by the EMP pathway and the PP pathway, with glucose uptake exclusively by the PTS.

1. Material balances around metabolic nodes (Determined System)

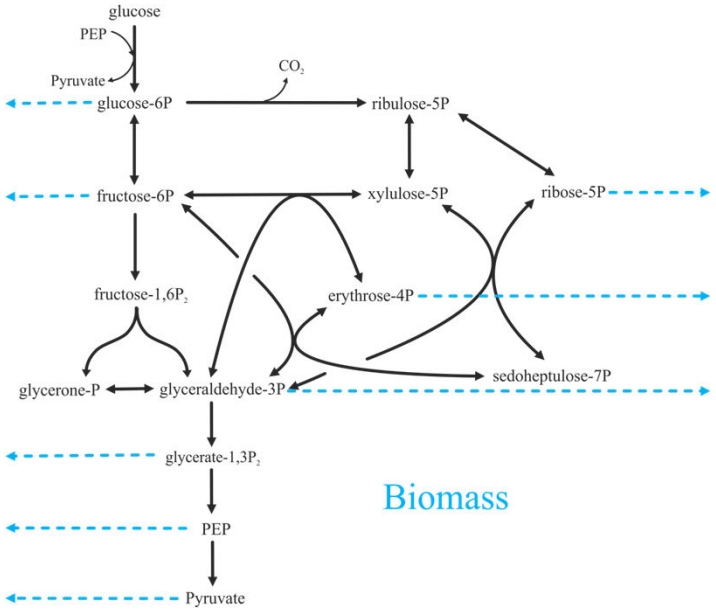


Steps:

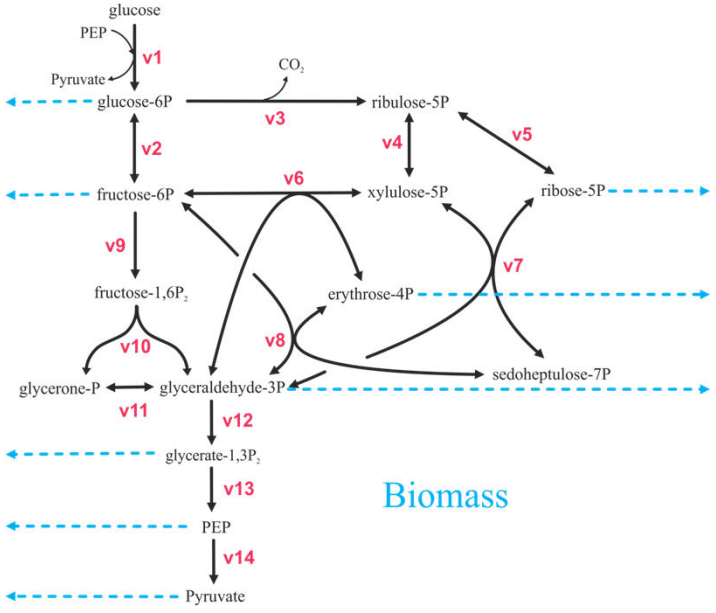
a) Draw complete proposed metabolic network.



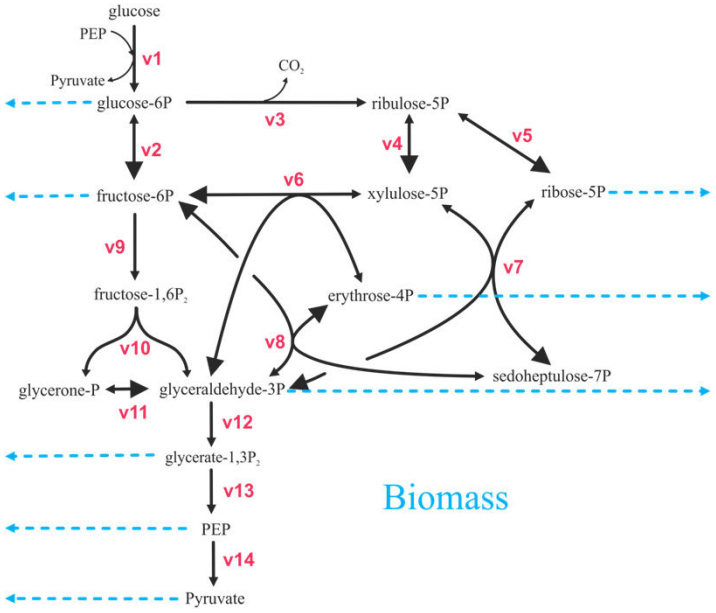
b) Simplify network to include only carbon, and only branch points.



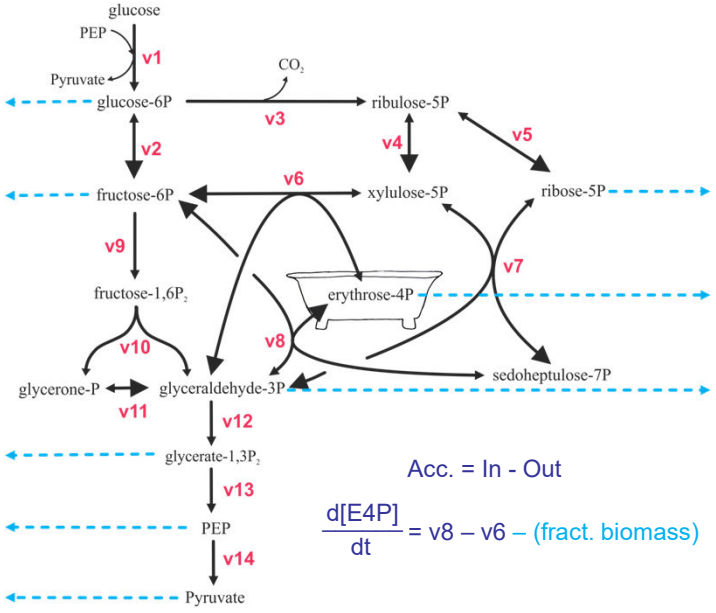
c) Assign a number to each flux and label network.



d) For reversible reactions, decide what is "forward" direction.



e) Write material balances around each node. (Erythrose-4P as example.)



Acc. = In - Out

$$\frac{d[E4P]}{dt} = v8 - v6 - (\text{fract. biomass})$$

e) Write material balances around each node
(Assume no growth, Biomass flux = 0)

$\frac{d[Glu]}{dt} = -v1$

$\frac{d[G6P]}{dt} = v1 - v2 - v3$

$\frac{d[Ru5P]}{dt} = v3 - v4 - v5$

$\frac{d[X5P]}{dt} = v4 - v6 - v7$

$\frac{d[R5P]}{dt} = v5 - v7$

$\frac{d[S7P]}{dt} = v7 - v8$

$\frac{d[E4P]}{dt} = v8 - v6$

$\frac{d[F6P]}{dt} = v2 + v6 + v8 - v9$

$\frac{d[FDP]}{dt} = v9 - v10$

$\frac{d[GlP]}{dt} = v10 - v11$

$\frac{d[G3P]}{dt} = v10 + v11 + v6 + v7 - v8 - v12$

$\frac{d[3PG]}{dt} = v12 - v13$

$\frac{d[PEP]}{dt} = v13 - v14 - v1$

$\frac{d[Pyr]}{dt} = v14 + v1$

f) Provide values for known fluxes.
Typically, product formation rate (we'll use v_{Pyr} = 3.41 mmol/gh) and substrate consumption rate (v_{Glu} = -1.78 mmol/gh) are known.
Steady-state assumption – the metabolic pools must not change.

$v_{Glu} = -v1$

$0 = v1 - v2 - v3$

$0 = v3 - v4 - v5$

$0 = v4 - v6 - v7$

$0 = v5 - v7$

$0 = v7 - v8$

$0 = v8 - v6$

$0 = v2 + v6 + v8 - v9$

$0 = v9 - v10$

$0 = v10 - v11$

$0 = v10 + v11 + v6 + v7 - v8 - v12$

$0 = v12 - v13$

$0 = v13 - v14 - v1$

$v_{Pyr} = v14 + v1$

g) Solve the equations (Note we have 14 equations and 14 unknowns).

1) Linear algebra

$$v_{Glu} = -1v1 + 0v2 + 0v3 + 0v4 + 0v5 + 0v6 + 0v7 + 0v8 + 0v9 + 0v10 + 0v11 + 0v12 + 0v13 + 0v14$$

$$0 = 1v1 - 1v2 - 1v3 + 0v4 + 0v5 + 0v6 + 0v7 + 0v8 + 0v9 + 0v10 + 0v11 + 0v12 + 0v13 + 0v14$$

$$0 = 0v1 + 0v2 + 1v3 - 1v4 - 1v5 + 0v6 + 0v7 + 0v8 + 0v9 + 0v10 + 0v11 + 0v12 + 0v13 + 0v14$$

$$0 = 0v1 + 0v2 + 0v3 + 1v4 + 0v5 - 1v6 - 1v7 + 0v8 + 0v9 + 0v10 + 0v11 + 0v12 + 0v13 + 0v14$$

$$0 = 0v1 + 0v2 + 0v3 + 0v4 + 1v5 + 0v6 - 1v7 + 0v8 + 0v9 + 0v10 + 0v11 + 0v12 + 0v13 + 0v14$$

$$0 = 0v1 + 0v2 + 0v3 + 0v4 + 0v5 + 0v6 + 1v7 - 1v8 + 0v9 + 0v10 + 0v11 + 0v12 + 0v13 + 0v14$$

$$0 = 0v1 + 0v2 + 0v3 + 0v4 + 0v5 - 1v6 + 0v7 + 1v8 + 0v9 + 0v10 + 0v11 + 0v12 + 0v13 + 0v14$$

$$0 = 0v1 + 1v2 + 0v3 + 0v4 + 0v5 + 1v6 + 0v7 + 1v8 - 1v9 + 0v10 + 0v11 + 0v12 + 0v13 + 0v14$$

$$0 = 0v1 + 0v2 + 0v3 + 0v4 + 0v5 + 0v6 + 0v7 + 0v8 + 1v9 - 1v10 + 0v11 + 0v12 + 0v13 + 0v14$$

$$0 = 0v1 + 0v2 + 0v3 + 0v4 + 0v5 + 0v6 + 0v7 + 0v8 + 0v9 + 1v10 - 1v11 + 0v12 + 0v13 + 0v14$$

$$0 = 0v1 + 0v2 + 0v3 + 0v4 + 0v5 + 1v6 + 1v7 - 1v8 + 0v9 + 1v10 + 1v11 - 1v12 + 0v13 + 0v14$$

$$0 = 0v1 + 0v2 + 0v3 + 0v4 + 0v5 + 0v6 + 0v7 + 0v8 + 0v9 + 0v10 + 0v11 + 1v12 - 1v13 + 0v14$$

$$0 = -1v1 + 0v2 + 0v3 + 0v4 + 0v5 + 0v6 + 0v7 + 0v8 + 0v9 + 0v10 + 0v11 + 0v12 + 1v13 - 1v14$$

$$v_{Pyr} = 1v1 + 0v2 + 0v3 + 0v4 + 0v5 + 0v6 + 0v7 + 0v8 + 0v9 + 0v10 + 0v11 + 0v12 + 0v13 + 1v14$$

g) Solve the equations...

1) Linear algebra

$$\mathbf{b = Av}$$

$$\mathbf{v = A^{-1}b}$$

$$v = \begin{bmatrix} v1 \\ v2 \\ v3 \\ v4 \\ v5 \\ v6 \\ v7 \\ v8 \\ v9 \\ v10 \\ v11 \\ v12 \\ v13 \\ v14 \end{bmatrix} \quad b = \begin{bmatrix} v_{Glu} \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ v_{Pyr} \end{bmatrix} = \begin{bmatrix} -1.78 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 3.41 \end{bmatrix} \quad A = \begin{bmatrix} -1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 1 & -1 & -1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 1 & -1 & -1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 1 & 0 & -1 & -1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 1 & 0 & -1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 1 & -1 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & -1 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 1 & 0 & 0 & 0 & 1 & 0 & 1 & -1 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & -1 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & -1 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 1 & 1 & -1 & 0 & 1 & 1 & -1 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & -1 & 0 \\ -1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & -1 \\ 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 \end{bmatrix}$$

MATLAB Program

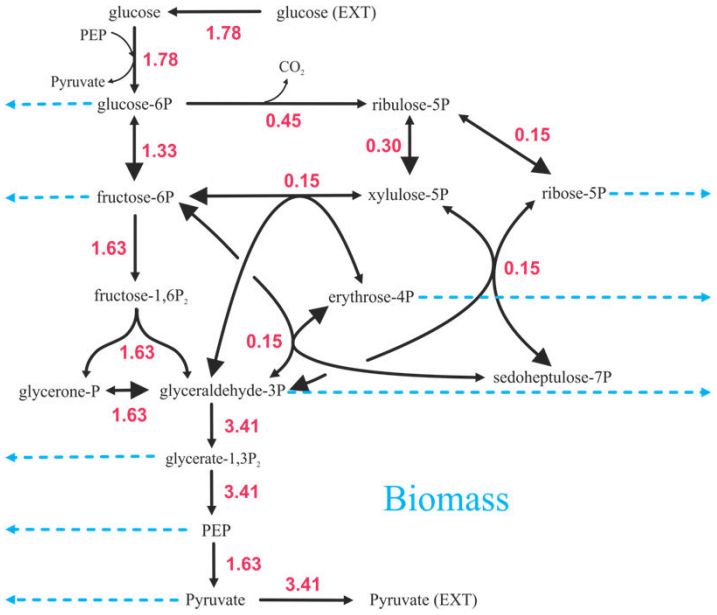
```
%% This MATLAB program solves a set of 14 linear equations.  They
must be consistent and non-singular!
%%

% define blank array
b = [0;0;0;0;0;0;0;0;0;0;0;0;0;0];
v = [0;0;0;0;0;0;0;0;0;0;0;0;0;0];

% define constants
b(1) = -1.78;
b(14) = 3.41;

A = [-1,0,0,0,0,0,0,0,0,0,0,0,0,0;
    1,-1,-1,0,0,0,0,0,0,0,0,0,0,0;
    0,0,1,-1,-1,0,0,0,0,0,0,0,0,0;
    0,0,0,1,0,-1,-1,0,0,0,0,0,0,0;
    0,0,0,0,1,0,-1,0,0,0,0,0,0,0;
    0,0,0,0,0,0,1,-1,0,0,0,0,0,0;
    0,0,0,0,0,-1,0,1,0,0,0,0,0,0;
    0,1,0,0,0,1,0,1,-1,0,0,0,0,0;
    0,0,0,0,0,0,0,0,1,-1,0,0,0,0;
    0,0,0,0,0,0,0,0,0,1,-1,0,0,0;
    0,0,0,0,0,1,1,-1,0,1,1,-1,0,0;
    0,0,0,0,0,0,0,0,0,0,1,-1,0;
    -1,0,0,0,0,0,0,0,0,0,0,0,1,-1;
    1,0,0,0,0,0,0,0,0,0,0,0,0,1];

v = A\b
```

$$v = \begin{bmatrix} v1 \\ v2 \\ v3 \\ v4 \\ v5 \\ v6 \\ v7 \\ v8 \\ v9 \\ v10 \\ v11 \\ v12 \\ v13 \\ v14 \end{bmatrix} = \begin{bmatrix} 1.78 \\ 1.33 \\ 0.45 \\ 0.30 \\ 0.15 \\ 0.15 \\ 0.15 \\ 0.15 \\ 1.63 \\ 1.63 \\ 1.63 \\ 3.41 \\ 3.41 \\ 1.63 \end{bmatrix}$$

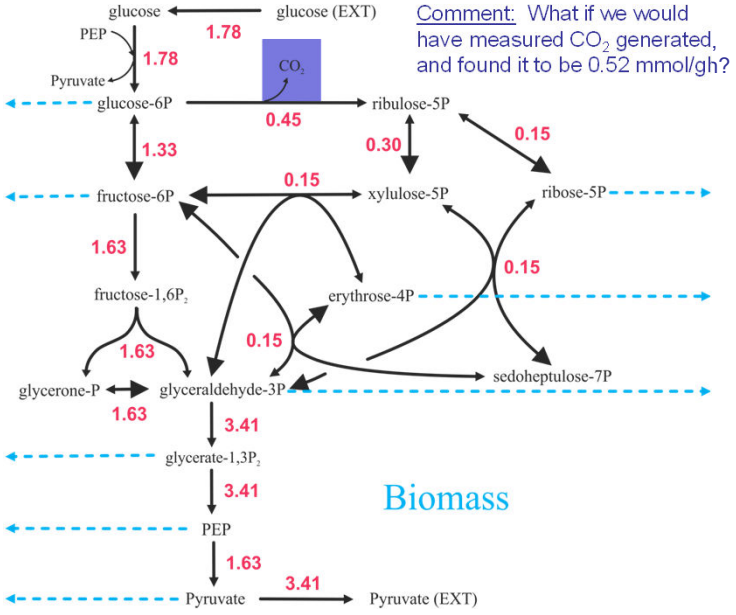
2) Just solve the equations straight away!

$v1 = v_{Glu}$	$v1 = 1.78$
$v2 = 3v_{Pyr} - 5v_{Glu}$	$v2 = 3(3.41) - 5(1.78) = 1.33$
$v3 = 6v_{Glu} - 3v_{Pyr}$	$v3 = 6(1.78) - 3(3.41) = 0.45$
$v4 = 4v_{Glu} - 2v_{Pyr}$	$v4 = 4(1.78) - 2(3.41) = 0.30$
$v5 = 2v_{Glu} - v_{Pyr}$	$v5 = 2(1.78) - 3.41 = 0.15$
$v6 = 2v_{Glu} - v_{Pyr}$	$v6 = 2(1.78) - 3.41 = 0.15$
$v7 = 2v_{Glu} - v_{Pyr}$	$v7 = 2(1.78) - 3.41 = 0.15$
$v8 = 2v_{Glu} - v_{Pyr}$	$v8 = 2(1.78) - 3.41 = 0.15$
$v9 = v_{Pyr} - v_{Glu}$	$v9 = 3.41 - 1.78 = 1.63$
$v10 = v_{Pyr} - v_{Glu}$	$v10 = 3.41 - 1.78 = 1.63$
$v11 = v_{Pyr} - v_{Glu}$	$v11 = 3.41 - 1.78 = 1.63$
$v12 = v_{Pyr}$	$v12 = 3.41$
$v13 = v_{Pyr}$	$v13 = 3.41$
$v14 = v_{Pyr} - v_{Glu}$	$v14 = 3.41 - 1.78 = 1.63$

h) Go back and take a look at the metabolic network. Does it make sense?



2. Material balances around metabolic nodes (Overdetermined System)



Comment: What if we would have measured CO_2 generated, and found it to be 0.52 mmol/gh?

a) If we know the CO_2 flux (v_{CO2}), then we can include a known CO_2 flux in our analysis.
When we write material balances around each node (step "e" in previous example), we introduce another equation representing a CO_2 mass balance.
Because we now have 15 equations and 14 unknowns, this system is overdetermined.

$v_{Glu} = -v1$

$v_{CO2} = v3$

$0 = v1 - v2 - v3$

$0 = v3 - v4 - v5$

$0 = v4 - v6 - v7$

$0 = v5 - v7$

$0 = v7 - v8$

$0 = v8 - v6$

$0 = v2 + v6 + v8 - v9$

$0 = v9 - v10$

$0 = v10 - v11$

$0 = v10 + v11 + v6 + v7 - v8 - v12$

$0 = v12 - v13$

$0 = v13 - v14 - v1$

$v_{Pyr} = v14 + v1$

This additional equation represents the (steady-state) balance around the CO_2 node.

b) Solve the equations (Note we have 15 equations and 14 unknowns).

1) For an overdetermined system, write the equations with the known fluxes on the right side of the equality, appearing first.

$0 = -1v_{Glu} + 0v_{Pyr} + 0v_{CO2} - 1v1 + 0v2 + 0v3 + 0v4 + 0v5 + 0v6 + 0v7 + 0v8 + 0v9 + 0v10 + 0v11 + 0v12 + 0v13 + 0v14$

$0 = 0v_{Glu} + 0v_{Pyr} - 1v_{CO2} + 0v1 - 0v2 + 1v3 + 0v4 + 0v5 + 0v6 + 0v7 + 0v8 + 0v9 + 0v10 + 0v11 + 0v12 + 0v13 + 0v14$

$0 = 0v_{Glu} + 0v_{Pyr} + 0v_{CO2} + 1v1 - 1v2 - 1v3 + 0v4 + 0v5 + 0v6 + 0v7 + 0v8 + 0v9 + 0v10 + 0v11 + 0v12 + 0v13 + 0v14$

$0 = 0v_{Glu} + 0v_{Pyr} + 0v_{CO2} + 0v1 + 0v2 + 1v3 - 1v4 - 1v5 + 0v6 + 0v7 + 0v8 + 0v9 + 0v10 + 0v11 + 0v12 + 0v13 + 0v14$

$0 = 0v_{Glu} + 0v_{Pyr} + 0v_{CO2} + 0v1 + 0v2 + 0v3 + 1v4 + 0v5 - 1v6 - 1v7 + 0v8 + 0v9 + 0v10 + 0v11 + 0v12 + 0v13 + 0v14$

$0 = 0v_{Glu} + 0v_{Pyr} + 0v_{CO2} + 0v1 + 0v2 + 0v3 + 0v4 + 1v5 + 0v6 - 1v7 + 0v8 + 0v9 + 0v10 + 0v11 + 0v12 + 0v13 + 0v14$

$0 = 0v_{Glu} + 0v_{Pyr} + 0v_{CO2} + 0v1 + 0v2 + 0v3 + 0v4 + 0v5 + 0v6 + 1v7 - 1v8 + 0v9 + 0v10 + 0v11 + 0v12 + 0v13 + 0v14$

$0 = 0v_{Glu} + 0v_{Pyr} + 0v_{CO2} + 0v1 + 0v2 + 0v3 + 0v4 + 0v5 - 1v6 + 0v7 + 1v8 + 0v9 + 0v10 + 0v11 + 0v12 + 0v13 + 0v14$

$0 = 0v_{Glu} + 0v_{Pyr} + 0v_{CO2} + 0v1 + 1v2 + 0v3 + 0v4 + 0v5 + 1v6 + 0v7 + 1v8 - 1v9 + 0v10 + 0v11 + 0v12 + 0v13 + 0v14$

$0 = 0v_{Glu} + 0v_{Pyr} + 0v_{CO2} + 0v1 + 0v2 + 0v3 + 0v4 + 0v5 + 0v6 + 0v7 + 0v8 + 1v9 - 1v10 + 0v11 + 0v12 + 0v13 + 0v14$

$0 = 0v_{Glu} + 0v_{Pyr} + 0v_{CO2} + 0v1 + 0v2 + 0v3 + 0v4 + 0v5 + 0v6 + 0v7 + 0v8 + 0v9 + 1v10 - 1v11 + 0v12 + 0v13 + 0v14$

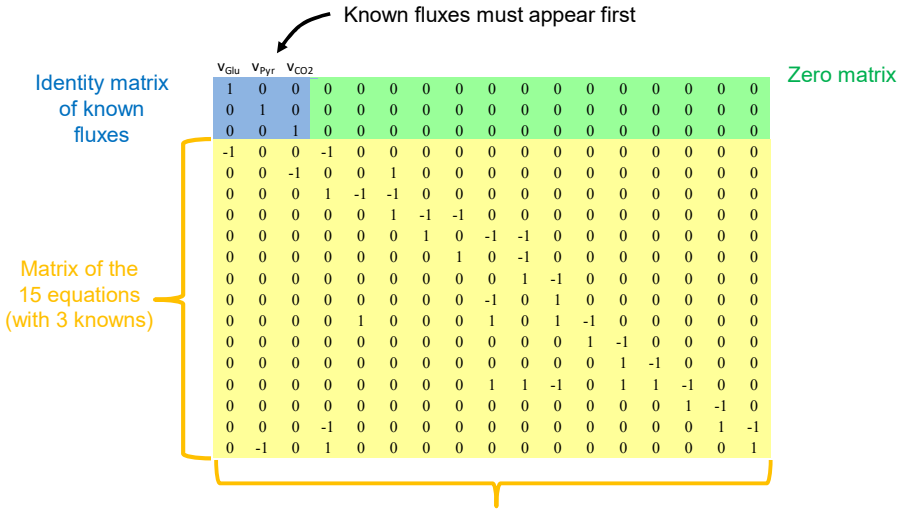
$0 = 0v_{Glu} + 0v_{Pyr} + 0v_{CO2} + 0v1 + 0v2 + 0v3 + 0v4 + 0v5 + 1v6 + 1v7 - 1v8 + 0v9 + 1v10 + 1v11 - 1v12 + 0v13 + 0v14$

$0 = 0v_{Glu} + 0v_{Pyr} + 0v_{CO2} + 0v1 + 0v2 + 0v3 + 0v4 + 0v5 + 0v6 + 0v7 + 0v8 + 0v9 + 0v10 + 0v11 + 1v12 - 1v13 + 0v14$

$0 = 0v_{Glu} + 0v_{Pyr} + 0v_{CO2} - 1v1 + 0v2 + 0v3 + 0v4 + 0v5 + 0v6 + 0v7 + 0v8 + 0v9 + 0v10 + 0v11 + 0v12 + 1v13 - 1v14$

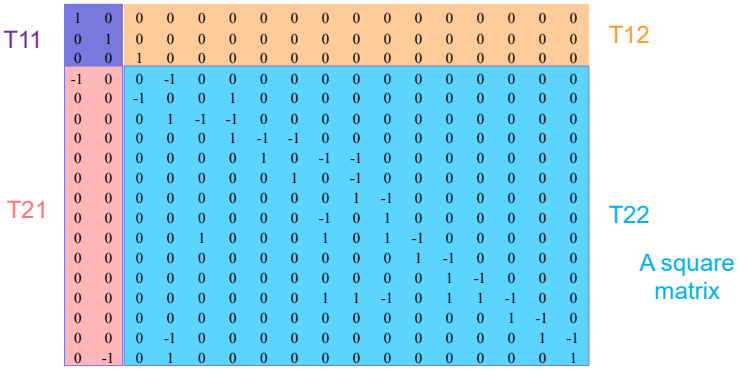
$0 = 0v_{Glu} - 1v_{Pyr} + 0v_{CO2} + 1v1 + 0v2 + 0v3 + 0v4 + 0v5 + 0v6 + 0v7 + 0v8 + 0v9 + 0v10 + 0v11 + 0v12 + 0v13 + 1v14$

2) Write a larger matrix from these equations:



v_{Glu}	v_{Pyr}	v_{CO2}														
1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
-1	0	0	-1	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	-1	0	0	1	0	0	0	0	0	0	0	0	0	0	0
0	0	0	1	-1	-1	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	1	-1	-1	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	1	0	-1	-1	0	0	0	0	0	0	0
0	0	0	0	0	0	0	1	0	-1	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	1	-1	0	0	0	0	0	0
0	0	0	0	0	0	0	0	-1	0	1	0	0	0	0	0	0
0	0	0	0	1	0	0	0	1	0	1	-1	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	1	-1	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	1	-1	0	0	0
0	0	0	0	0	0	0	0	1	1	-1	0	1	1	-1	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	-1	0
0	0	0	-1	0	0	0	0	0	0	0	0	0	0	0	1	-1
0	-1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1

3) Construct four new matrices from larger matrix:



1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
-1	0	0	-1	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	-1	0	0	1	0	0	0	0	0	0	0	0	0	0	0
0	0	0	1	-1	-1	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	1	-1	-1	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	1	0	-1	-1	0	0	0	0	0	0	0
0	0	0	0	0	0	0	1	0	-1	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	1	-1	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	-1	0	1	0	0	0	0	0	0
0	0	0	0	1	0	0	0	1	0	1	-1	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	1	-1	0	0	0	0
0	0	0	0	0	0	0	0	1	1	-1	0	1	1	-1	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	-1	0
0	0	0	-1	0	0	0	0	0	0	0	0	0	0	0	1	-1
0	-1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1

4) Solve the equations with the following algorithm:

$$T = T11 - T12*P(T22)*T21$$

$$v1 = P(T^T T) * T^T * vM$$

$$v2 = -P(T22) * T21 * v1$$

$$vM = \begin{bmatrix} v_{Glu} \\ v_{Pyr} \\ v_{CO2} \end{bmatrix} = \begin{bmatrix} -1.78 \\ 3.41 \\ 0.52 \end{bmatrix} \qquad v1 = \begin{bmatrix} v_{Glu} \\ v_{Pyr} \end{bmatrix} \qquad v2 = \begin{bmatrix} v_{CO2} \\ v1 \\ v2 \\ v3 \\ v4 \\ v5 \\ v6 \\ v7 \\ v8 \\ v9 \\ v10 \\ v11 \\ v12 \\ v13 \\ v14 \end{bmatrix}$$

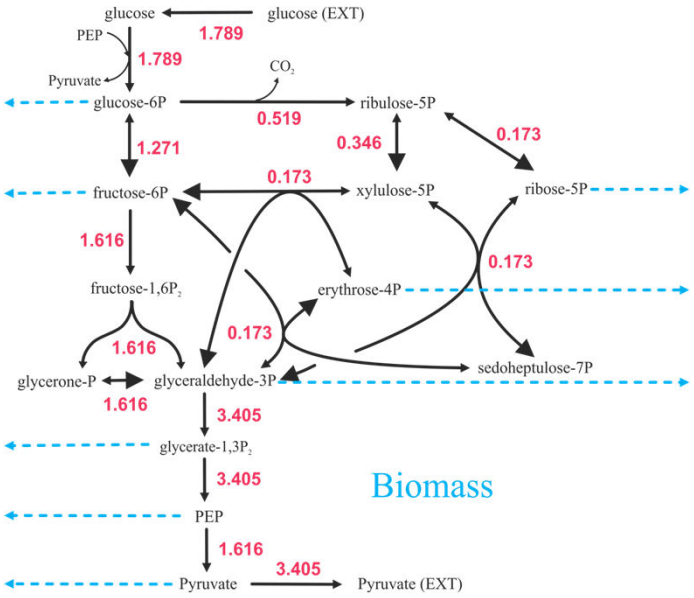
MATLAB Program

$$v1 = \begin{bmatrix} v_{Glu} \\ v_{Pyr} \end{bmatrix} = \begin{bmatrix} -1.789 \\ 3.405 \end{bmatrix}$$

$$v2 = \begin{bmatrix} v_{CO2} \\ v1 \\ v2 \\ v3 \\ v4 \\ v5 \\ v6 \\ v7 \\ v8 \\ v9 \\ v10 \\ v11 \\ v12 \\ v13 \\ v14 \end{bmatrix} = \begin{bmatrix} 0.519 \\ 1.789 \\ 1.271 \\ 0.519 \\ 0.346 \\ 0.173 \\ 0.173 \\ 0.173 \\ 1.616 \\ 1.616 \\ 1.616 \\ 3.405 \\ 3.405 \\ 3.405 \\ 1.616 \end{bmatrix}$$

```
%% This MATLAB program solves a set of 15 linear equations with
14 unknowns (overdetermined).
%%

%define matrices

T11 = [1,0;
       0,1;
       0,0];

T21 = [-1,0;
        0,0;
        0,0;
        0,0;
        0,0;
        0,0;
        0,0;
        0,0;
        0,0;
        0,0;
        0,0;
        0,0;
        0,0;
        0,0;
        0,-1];

T12 = [0,0,0,0,0,0,0,0,0,0,0,0,0,0;
       0,0,0,0,0,0,0,0,0,0,0,0,0,0;
       1,0,0,0,0,0,0,0,0,0,0,0,0,0];

T22 = [0,-1,0,0,0,0,0,0,0,0,0,0,0,0;
       -1,0,0,1,0,0,0,0,0,0,0,0,0,0;
        0,1,-1,-1,0,0,0,0,0,0,0,0,0,0;
        0,0,0,1,-1,-1,0,0,0,0,0,0,0,0;
        0,0,0,0,1,0,-1,-1,0,0,0,0,0,0;
        0,0,0,0,0,1,0,-1,0,0,0,0,0,0;
        0,0,0,0,0,0,0,1,-1,0,0,0,0,0;
        0,0,0,0,0,0,0,0,1,-1,0,0,0,0;
        0,0,1,0,0,0,1,0,1,-1,0,0,0,0;
        0,0,0,0,0,0,0,0,0,1,-1,0,0,0;
        0,0,0,0,0,0,0,0,0,0,1,-1,0,0;
        0,0,0,0,0,0,0,1,1,-1,0,1,1,-1;
        0,1,0,0,0,0,0,0,0,0,0,0,0,1];

vM = [-1.78;3.41;0.52];

%the algorithm

T = T11 - T12*pinv(T22)*T21;
TT = transpose(T);
v1 = pinv(TT*T)*TT*vM
v2 = -pinv(T22)*T21*v1
```

c) Go back and take a look at the metabolic network.



3. Material balances around metabolic nodes and include biomass formation

a) Need to known how much of each "precursor" is needed for cells in units of mol precursor/mol cell (stoichiometric coefficient).

To include biomass formation in the material balance at step "e", we need to know how much of each precursor molecule contributes to biomass, (and what is the rate of biomass formation from the experiment). Precursor information is often available in literature.

Using *E. coli* as an example, imagine an experiment in which we determine the amount of each of the amino acids present in typical *E. coli* cell. By knowing the biochemical pathways *E. coli* uses to generate each amino acid from "precursors", we can determine how much of each precursor is needed.

		Precursor Molecule											Other molecules					
Amino Acid Building Block	Amount Present in *E. coli* (μmol/g cells)	acetyl CoA	erythrose 4-P	fructose 6-P	glucose 6-P	a-ketoglutarate	oxaloacetate	ribose 5-P	PEP	3-phosphiglycerate	pyruvate	dihydroxyacetone-P	ATP	NADH	NADPH	1-C	NH4	S
Alanine	488										1				1		1	
Arginine	281					1							7	-1	4		4	
Asparagine	229						1						3		1		2	
Aspartate	229						1								1		1	
Cysteine	87									1			4	-1	5		1	1
Glutamate	250					1									1		1	
Glutamine	250					1							1		1		2	
Glycine	582									1				-1	1	-1	1	
Histidine	90							1					6	-3	1	1	3	
Isoleucine	276						1				1		2		5		1	
Leucine	428	1									2			-1	2		1	
Lysine	326						1				1		2		4		2	
Methionine	146						1						7		8	1	1	1
Phenylalanine	176		1						2				1		2		1	
Proline	210					1							1		3		1	
Serine	205									1				-1	1		1	
Threonine	241						1						2		3		1	
Tryptophan	54		1					1	1				5	-2	3		2	
Tyrosine	131		1						2				1	-1	2		1	
Valine	402										2				2		1	

Neidhardt et al. 1990, p. 135.

Example (see Table): To generate 1 g of cells, 176 μmol phenylalanine must be generated. This quantity of phenylalanine will require the following (stoichiometric) quantities of specific precursors:

176 μmol erythrose-4P
352 μmol PEP
176 μmol ATP
352 μmol NADPH
176 μmol NH_4^+

		Precursor Molecule												Other molecules					
Amino Acid Building Block	Amount Present in *E. coli* (μmol/g cells)	acetyl CoA	erythrose 4-P	fructose 6-P	glucose 6-P	a-ketoglutarate	oxaloacetate	ribose 5-P	PEP	3-phosphglycerate	pyruvate	dihydroxyacetone-P	ATP	NADH	NADPH	1-C	NH4	S
Alanine	488										1				1		1	
Arginine	281					1							7	-1	4		4	
Asparagine	229						1						3		1		2	
Aspartate	229						1								1		1	
Cysteine	87									1			4	-1	5		1	1
Glutamate	250					1									1		1	
Glutamine	250					1							1		1		2	
Glycine	582									1				-1	1	-1	1	
Histidine	90							1					6	-3	1	1	3	
Isoleucine	276						1				1		2		5		1	
Leucine	428	1									2			-1	2		1	
Lysine	326						1				1		2		4		2	
Methionine	146						1						7		8	1	1	1
Phenylalanine	176		1						2				1		2		1	
Proline	210					1							1		3		1	
Serine	205									1				-1	1		1	
Threonine	241						1						2		3		1	
Tryptophan	54		1					1	1				5	-2	3		2	
Tyrosine	131		1						2				1	-1	2		1	
Valine	402										2				2		1	

Neidhardt et al. 1990, p. 135.

We can also use this table to calculate the total amount of each precursor needed for a gram of cells. For example (see Table): To generate the protein needed for 1 g of cells, the following quantity of erythrose-4P is needed:

176 μmol (for phenylalanine)
54 μmol (for tryptophan)
131 μmol (for tyrosine)
361 μmol erythrose-4P needed to generate protein in 1 g of cells

Since erythrose-4P is only needed for proteins in cells:

361 μmol erythrose-4P/g cells

b) Calculating stoichiometric coefficient

	precursors needed (μmol/g cell)
G6P	205
F6P	70.9
R5P	897.7
E4P	361
Gly3P	129
3PG	1496
PEP	519
PYR	2832.8
AceCoA	3747.8
OKG	1078.9
OAA	1786.7

Unit carbon molecular weight of *E. coli*

need stoichiometric coefficient:

$$\frac{361\ \mu mol\ E4P}{g\ cells} \times \frac{24.70\ g\ cells}{mol\ cells} \times \frac{mol}{10^6\ \mu mol}$$

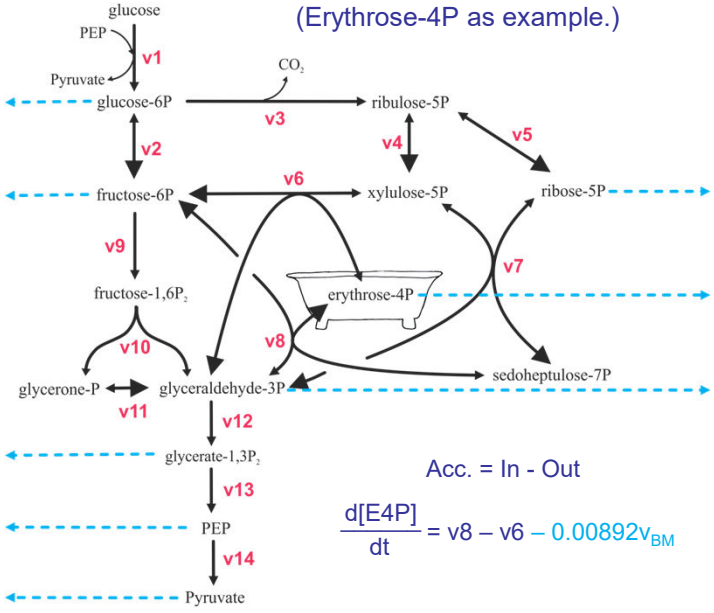
= 0.00892 mol E4P/mol cells

We will use v_{BM} as flux to biomass (mmol biomass/gh)

Neidhardt et al. 1990

c) Write material balances around each node (like before).

(Erythrose-4P as example.)



Acc. = In - Out

$$\frac{d[E4P]}{dt} = v8 - v6 - 0.00892 v_{BM}$$

c) Write material balances around each node

$$\frac{d[Glu]}{dt} = -v1$$

$$\frac{d[G6P]}{dt} = v1 - v2 - v3 - 0.00506v_{BM}$$

$$\frac{d[Ru5P]}{dt} = v3 - v4 - v5$$

$$\frac{d[X5P]}{dt} = v4 - v6 - v7$$

$$\frac{d[R5P]}{dt} = v5 - v7 - 0.0222v_{BM}$$

$$\frac{d[S7P]}{dt} = v7 - v8$$

$$\frac{d[E4P]}{dt} = v8 - v6 - 0.00892v_{BM}$$

$$\frac{d[F6P]}{dt} = v2 + v6 + v8 - v9 - 0.00175v_{BM}$$

$$\frac{d[FDP]}{dt} = v9 - v10$$

$$\frac{d[GlP]}{dt} = v10 - v11$$

$$\frac{d[G3P]}{dt} = v10 + v11 + v6 + v7 - v8 - v12 - 0.00319v_{BM}$$

$$\frac{d[3PG]}{dt} = v12 - v13 - 0.0370v_{BM}$$

$$\frac{d[PEP]}{dt} = v13 - v14 - v1 - 0.0128v_{BM}$$

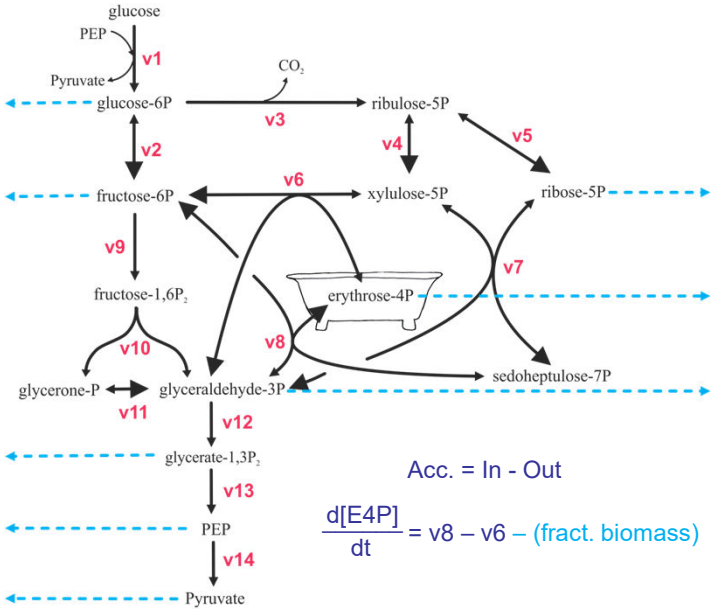
$$\frac{d[Pyr]}{dt} = v14 + v1 - 0.0700v_{BM}$$

then continue as before...

4. Material balances around metabolic nodes and include carbon position balance (for ^{13}C labeled experiments)

Not only do molecules each satisfy a material balance, but each carbon atom satisfies a material balance. So, for example, the C-1 carbon on erythrose-4P came from specific carbon atoms on other molecules when erythrose-4P was generated, and the C-1 carbon on erythrose-4P will be transferred to specific carbon atoms on other molecules when erythrose-4P is consumed. Thus, we can complete a carbon balance on each carbon atom in metabolism. Doing this type of balance is only helpful if we are able to distinguish carbon atoms, for example by using ^{13}C labeled substrates, and measuring the concentration of each atom in solution.

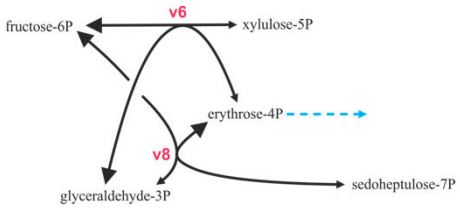
a) Complete a carbon (atom-by-atom) balance (Erythrose-4P as example.)



Acc. = In - Out

$$\frac{d[E4P]}{dt} = v8 - v6 - (\text{fract. biomass})$$

b) Focus on erythrose-4P node



$v6 = v6^F - v6^R$
$v8 = v8^F - v8^R$

F = forward conversion
R = reverse conversion

Often you see term exchange rate

$\zeta 6$ = exchange rate for flux 6 = $v6^R = v6^F - v6$
$\zeta 8$ = exchange rate for flux 8 = $v8^R = v8^F - v8$

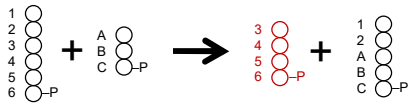
c) Write all equations involving erythrose-4P (Biomass flux = 0)

F6P + Gly3P → E4P + Xu5P ($v6^R$)
E4P + Xu5P → F6P + Gly3P ($v6^F$)
S7P + Gly3P → E4P + F6P ($v8^F$)
E4P + F6P → S7P + Gly3P ($v8^R$)

d) Write carbon atoms involved in these conversions

$v6^R$

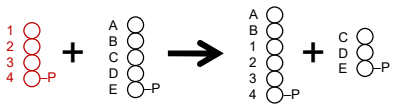
F6P + Gly3P → E4P + Xu5P



Numbers/letters correspond to F6P & Gly3P carbons!

$v6^F$

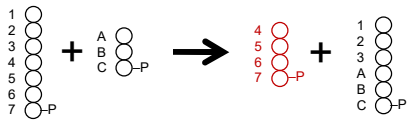
E4P + Xu5P → F6P + Gly3P



Numbers/letters correspond to E4P & Xu5P carbons!

$v8^F$

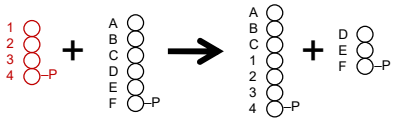
S7P + Gly3P → E4P + F6P



Numbers/letters correspond to S7P & Gly3P carbons!

$v8^R$

E4P + F6P → S7P + Gly3P



Numbers/letters correspond to E4P & F6P carbons!

e) Write material balance for each carbon atom
(example C-1 on erythrose-4P)

Define:

C_{E4P} = concentration of erythrose-4P
$C_{E4P(1)}$ = concentration of C-1 erythrose-4P = C_{E4P}
$C_{E4P(1)^*}$ = concentration of erythrose-4P labeled at C-1

$f_{E4P(1)^*}$ = fractional (label) enrichment at C-1 = $C_{E4P(1)^*}/C_{E4P}$

Recall:

E4P(1) comes from F6P(3) via $v6^R$
E4P(1) comes from S7P(4) via $v8^F$
E4P(1) goes to F6P(3) via $v6^F$
E4P(1) goes to S7P(4) via $v8^R$

So:

$$\frac{d[C_{E4P(1)^*}]}{dt} = f_{F6P(3)^*}v6^R + f_{S7P(4)^*}v8^F - f_{E4P(1)^*}v6^F - f_{E4P(1)^*}v8^R$$

Assumptions:

1) Assume metabolic and isotopic steady-state!
2) Change notation: let $f_{E4P(1)^*}$ = E4P(1), etc.

Then:

$$0 = F6P(3)\cdot v6^R + S7P(4)\cdot v8^F - E4P(1)\cdot v6^F - E4P(1)\cdot v8^R$$

Write analogous material balance for each carbon (on E4P):

C-1: $0 = F6P(3)\cdot v6^R + S7P(4)\cdot v8^F - E4P(1)\cdot v6^F - E4P(1)\cdot v8^R$

C-2: $0 = F6P(4)\cdot v6^R + S7P(5)\cdot v8^F - E4P(2)\cdot v6^F - E4P(2)\cdot v8^R$

C-3: $0 = F6P(5)\cdot v6^R + S7P(6)\cdot v8^F - E4P(3)\cdot v6^F - E4P(3)\cdot v8^R$

C-4: $0 = F6P(6)\cdot v6^R + S7P(7)\cdot v8^F - E4P(4)\cdot v6^F - E4P(4)\cdot v8^R$

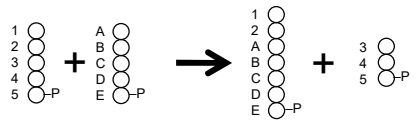
Or, in Matrix Form:

$$\mathbf{0} = \begin{pmatrix} F6P(3) \\ F6P(4) \\ F6P(5) \\ F6P(6) \end{pmatrix} v6^R + \begin{pmatrix} S7P(4) \\ S7P(5) \\ S7P(6) \\ S7P(7) \end{pmatrix} v8^F - \begin{pmatrix} E4P(1) \\ E4P(2) \\ E4P(3) \\ E4P(4) \end{pmatrix} v6^F - \begin{pmatrix} E4P(1) \\ E4P(2) \\ E4P(3) \\ E4P(4) \end{pmatrix} v8^R$$

Two other conversion involve alteration of carbon positions:

$v7^F$

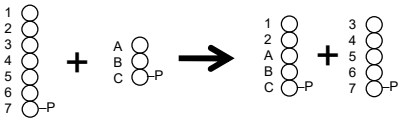
X5P + R5P → S7P + Gly3P



Numbers/letters correspond to X5P & R5P carbons!

$v7^R$

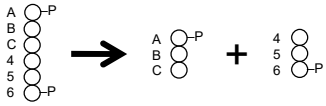
S7P + Gly3P → X5P + R5P



Numbers/letters correspond to S7P & Gly3P carbons!

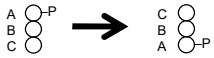
v10

F6P → GlyP + Gly3P



v11

GlyP → Gly3P



Numbers/letters correspond to F6P carbons!

C-1 → C-3
C-2 → C-2
C-3 → C-1
C-4 → C-1
C-5 → C-2
C-6 → C-3

f) Typical algorithm

- Often one “guesses” a value for partitioning at a node, and one “guesses” a value for each exchange rate.
- Carry the calculation “forward”
- Compare the resulting calculated enrichment of the product or an intermediate with the observed enrichment.
- Minimize the error between the calculated enrichment and the observed enrichment.

$$\text{Error} = \sum_{i=1}^{Each\ Carbon} \left(\frac{P_{OBS}(i) - P_{CALC}(i)}{P_{OBS}(i)} \right)^2$$

Where P is the product or intermediate.

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