Metabolic Flux Analysis in Cell Culture

By Wei-Shou Hu

I. OVERALL MATERIAL BALANCE FOR REACTION SYSTEMS

A. Chemical reaction systems

Metabolic flux analysis is the balance of material flow in cellular metabolism. Since mass is not created or destroyed in reactions, and only chemical conversion occurs, the nutrients taken up by cells (glucose, amino acids, oxygen, other organic and inorganic compounds) must all be accounted for (as new biomass, excreted metabolites (lactate, amino acids, CO_2, H_2O) and product). The idea is to use the overall balances on these inputs (nutrients) and outputs (new biomass, metabolites, products) to gain insight into the detailed view of the reaction occurring inside the cells. The methodology is analogous to solving a typical chemical reaction problem.

Example: Incomplete combustion of CH_4 to CO_2, CO and H_2O.

Input $CH_4 + O_2 \rightarrow$

$$\boxed{\begin{aligned} CH_4 + 2O_2 &\xrightarrow{\xi} CO_2 + 2H_2O \\ 2CH_4 + 3O_2 &\xrightarrow{1-\xi} 2CO + 4H_2O \end{aligned}}$$

Known Reactions

Output $\rightarrow CO_2 + CO + H_2O$

If all the inputs and outputs (i.e., the amount of CH_4, O_2 consumed and that of CO_2, CO, H_2O produced) are completely balanced, the fraction of CH_4 going to both reactions can be determined. On the other hand, if the material balance is not closed, there will be uncertainty about the solution, and the distribution of materials can only be estimated.

Case I. 4 moles of CH_4 and 7 moles of O_2 are combusted to produce 2 moles each of CO_2 and CO and 8 moles of H_2O.

In this case, all three elements involved—C, H and O—are completely balanced. The only unknown ξ can be easily calculated by balance on any elemental balance, e.g., use C balance,

$$\left(4 \text{ mole } CH_4 \times 1 \frac{\text{mole C}}{\text{mole } CH_4}\right) \times \xi = 2 \text{ mole } CO_2 \times 1 \frac{\text{mole C}}{\text{mole } CO_2}$$

$$\xi = 0.5$$

Comment: In Case I, in principal, one does not need to know the quantities of all inputs and outputs to find the solution. From three elemental balances (C, H, O), we can have three linear equations. As long as there are only three unknowns, we can also solve for ξ. For example, even if the quantity of CO and H_2O are not measured, the solution will be the same.

However, in the real world, there is always a high degree of uncertainty about the accuracy of measurement; the overall balance is always an important check of the validity of the results.

Case II: 4 moles of CH_4 and 7 moles of O_2 are combusted to form 1.5 moles of CO_2 and 1.6 moles of CO_2. The amount of H_2O produced is not measured. Determine the fraction of methane completed combusted.

In this case, the input and output materials are not balanced. There are a total of 4 moles of C combusted but only 3.1 moles are accounted for in the products. Regardless of whether H_2O is measured, this presents a problem.

The cause of materials balance not being closed is not always known. Maybe it is due to measurement error, maybe there are other reactions going on and producing other products that are not accounted for. In this case, we can also get a plausible answer of how much CH_4 goes to complete and incomplete combustion. If we know the extent of various measurement errors and the amount of H_2O, we can get a more reliable estimate.

$$CH_4, O_2 \rightarrow \begin{bmatrix} 2CH_4 + 3O_2 \xrightarrow{J_1} 2CO + 4H_2O \\ CO + \frac{1}{2} O_2 \xrightarrow{J_2} CO_2 \end{bmatrix} \rightarrow CO, CO_2, H_2O$$

1. A GENERAL APPROACH TO FINDING A SOLUTION TO THIS EXAMPLE

Set up material balance equations for every species inside the furnace

Use q to represent external fluxes measured

$$\frac{dCH_4}{dt} = q_{CH_4} - 2J_1$$

$$\frac{dO_2}{dt} = q_{O_2} - 3J_1 - 0.5J_2$$

$$\frac{dCO}{dt} = -q_{CO} + 2J_1 - J_2$$

$$\frac{dCO_2}{dt} = -q_{CO_2} + J_2$$

$$\frac{dH_2O}{dt} = -q_{H_2O} + 4J_1$$

If we assume pseudo-steady state, all the left hand sides of the equations become zero. The equations thus become:

$$
\begin{aligned}
2J_1 &= q_{CH_4} \\
3J_1 + 0.5J_2 &= q_{O_2} \\
2J_1 - J_2 &= q_{CO} \\
J_2 &= q_{CO_2} \\
4J_1 &= q_{H_2O}
\end{aligned}
$$

Written in matrix form:

$$
\begin{bmatrix} 2 & 0 \\ 3 & 0.5 \\ 2 & -1 \\ 0 & 1 \\ 4 & 0 \end{bmatrix} \begin{bmatrix} J_1 \\ J_2 \end{bmatrix} = \begin{bmatrix} q_{CH_4} \\ q_{O_2} \\ q_{CO} \\ q_{CO_2} \\ q_{H_2O} \end{bmatrix}
$$

There are five equations. Depending on how many of the q's are measured, there may be a different number of unknowns (in addition to J_1 and J_2). The system may be overspecified (more equations than unknowns), underspecified (more unknowns than equations), or have a unique solution (the same number of equations as unknowns). In general, for biological metabolic reactions, the system is overspecified.

B. Metabolic system

1. LIMITATIONS OF MFA

Metabolic flux analysis is a material balance on biological metabolic systems. The general premise is to take all the inputs and outputs to try to solve a system of equations set up according to the

internal metabolic reactions. Intrinsically it has the following limitations:

a) Material balance is seldom closed

(1) Unmeasured species in input

Many cultures use complex medium (e.g., serum, primatone), making quantitation extremely difficult. Serum contributes to cell growth substantially by providing lipids, fatty acids, peptides, etc.

(2) Unmeasured species in output

H_2O and CO_2 are major metabolites, one (H_2O) cannot be measured in a reactor, the other (CO_2) is very difficult to quantify (one needs to do both gas and liquid phase) precisely. Some excreted species (such as excreted vesicles) are not usually measured.

b) Biomass estimation is imprecise

Another output, biomass, also poses major obstacles since its composition (either elemental composition or the content of protein, lipid, nucleic acids) is hard to measure and varies with cultivation method, physiology and culture time.

c) Large measurement error in the input and output rate.

Cell culture medium consists of a large number of compounds (amino acid, glucose, fatty acids, etc.) in small quantities. The measurement errors accumulate to a relatively large value.

d) Large reaction network needs to be simplified

e) Difficulty in energy and H balance

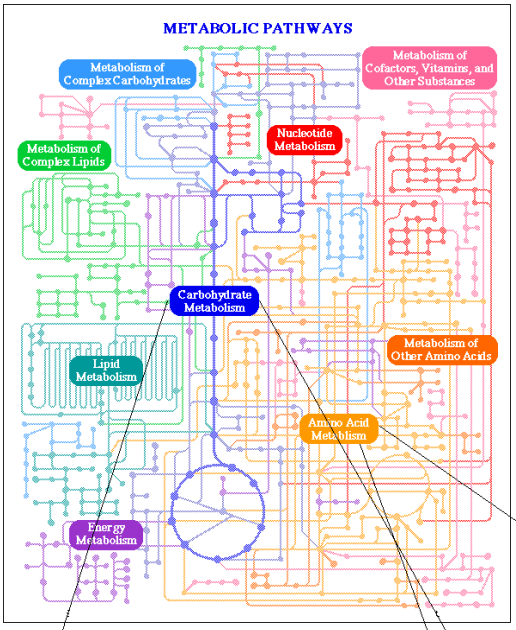
For mammalian cells, MFA is largely confined to C and N balances.

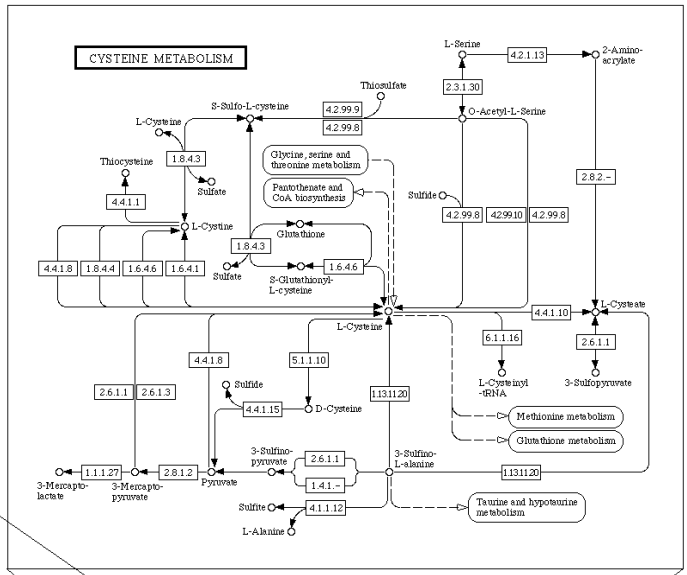
Mammalian cells have extensive membraneous structure. Nutrient and metabolite transport and maintenance of membrane potential incurs major energetic costs. These energetic costs contribute to a major part of O and H (via NADH, NADPH, ATP) balance, but they are difficult to account for.

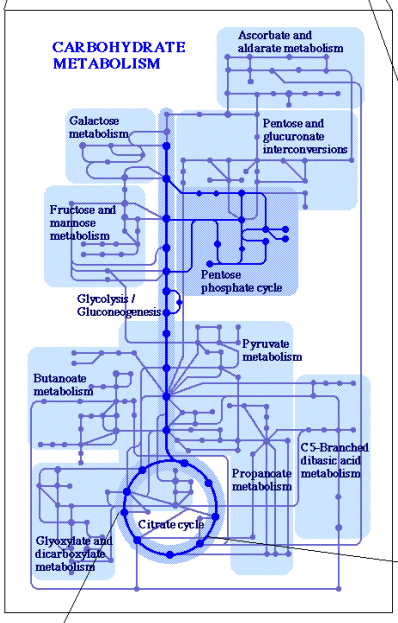
II. A SYSTEMATIC APPROACH TO METABOLIC FLUX ANALYSIS

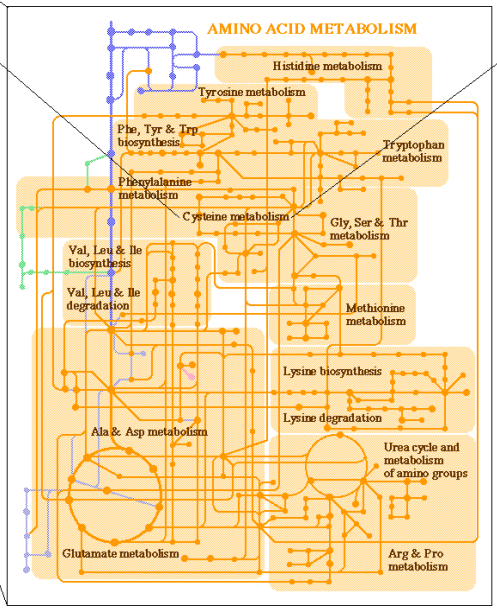
A. Establishing metabolic network

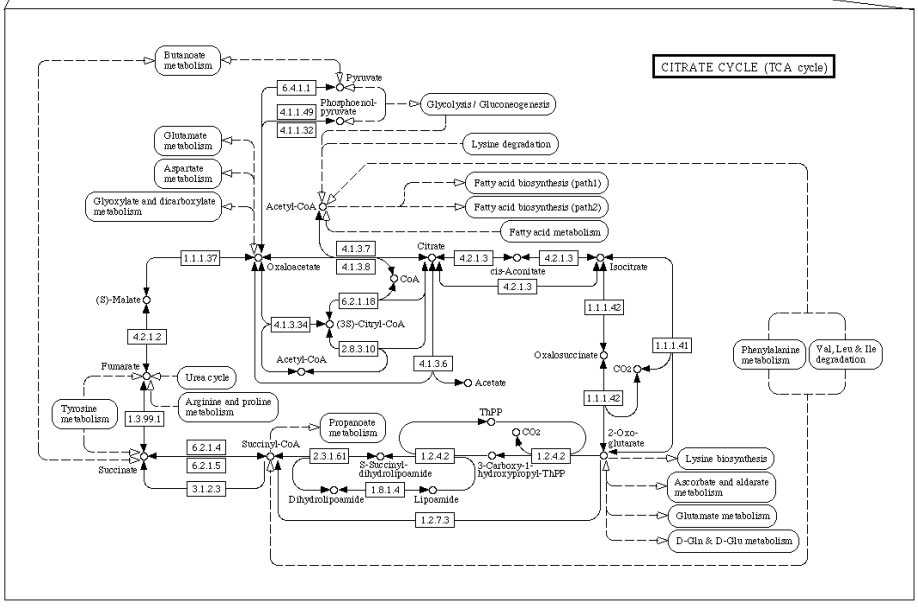
Identification of metabolic map and use of internet resources
(e.g., http://www.genome.ad.jp/kegg/metabolism.html)


METABOLIC PATHWAYS
Metabolism of Complex Carbohydrates
Metabolism of Cofactors, Vitamins, and Other Substances
Nucleotide Metabolism
Metabolism of Complex Lipids
Carbohydrate Metabolism
Metabolism of Other Amino Acids
Lipid Metabolism
Amino Acid Metabolism
Energy Metabolism


CYSTEINE METABOLISM
L-Serine
2-Amino-acrylate
Thiosulfate
O-Acetyl-L-Serine
S-Sulfo-L-cysteine
L-Cysteine
Thiocysteine
Sulfate
Glycine, serine and threonine metabolism
Pantothenate and CoA biosynthesis
Sulfide
L-Cystine
Glutathione
S-Glutathionyl-L-cysteine
L-Cysteine
L-Cysteinyl-tRNA
3-Sulfopyruvate
Sulfide
D-Cysteine
Methionine metabolism
Glutathione metabolism
3-Sulfino-pyruvate
3-Sulfino-L-alanine
3-Mercapto-lactate
3-Mercapto-pyruvate
Pyruvate
Sulfite
L-Alanine
Taurine and hypotaurine metabolism


CARBOHYDRATE METABOLISM
Ascorbate and aldarate metabolism
Galactose metabolism
Pentose and glucuronate interconversions
Fructose and mannose metabolism
Pentose phosphate cycle
Glycolysis / Gluconeogenesis
Pyruvate metabolism
Butanoate metabolism
C5-Branched dibasic acid metabolism
Propanoate metabolism
Citrate cycle
Glyoxylate and dicarboxylate metabolism

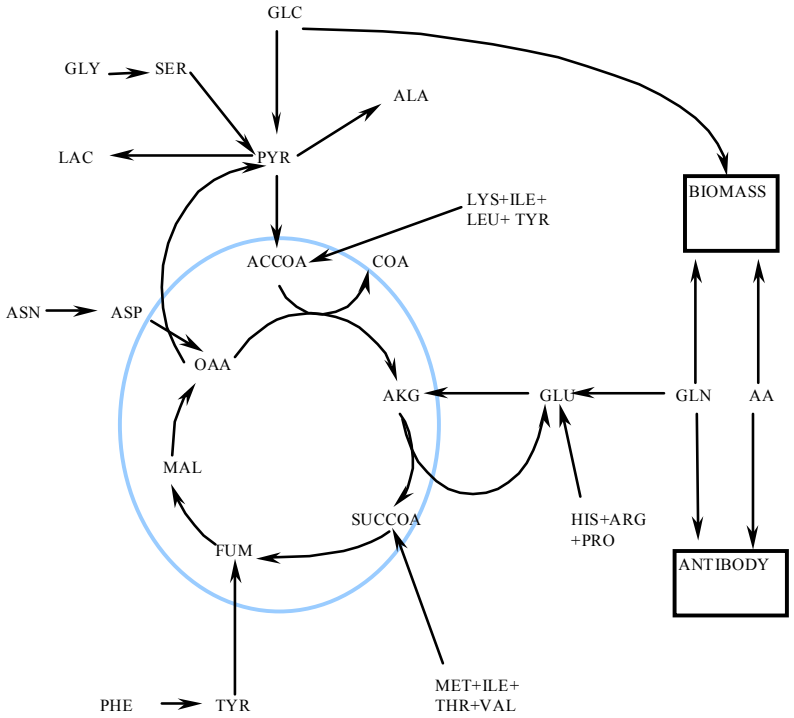

AMINO ACID METABOLISM
Histidine metabolism
Tyrosine metabolism
Phe, Tyr & Trp biosynthesis
Tryptophan metabolism
Phenylalanine metabolism
Cysteine metabolism
Gly, Ser & Thr metabolism
Val, Leu & Ile biosynthesis
Val, Leu & Ile degradation
Methionine metabolism
Lysine biosynthesis
Lysine degradation
Ala & Asp metabolism
Urea cycle and metabolism of amino groups
Glutamate metabolism
Arg & Pro metabolism


CITRATE CYCLE (TCA cycle)
Butanoate metabolism
Pyruvate
Phosphoenol-pyruvate
Glycolysis / Gluconeogenesis
Glutamate metabolism
Lysine degradation
Aspartate metabolism
Fatty acid biosynthesis (path1)
Glyoxylate and dicarboxylate metabolism
Acetyl-CoA
Fatty acid biosynthesis (path2)
Fatty acid metabolism
Citrate
Oxaloacetate
CoA
cis-Aconitate
Isocitrate
(S)-Malate
(3S)-Citryl-CoA
Oxalosuccinate
Fumarate
Urea cycle
Acetyl-CoA
Acetate
Phenylalanine metabolism
Val, Leu & Ile degradation
Tyrosine metabolism
Arginine and proline metabolism
ThPP
Propanoate metabolism
2-Oxo-glutarate
Succinyl-CoA
Lysine biosynthesis
Succinate
S-Succinyl-dihydrolipoamide
3-Carboxy-1-hydroxypropyl-ThPP
Ascorbate and aldarate metabolism
Dihydrolipoamide
Lipoamide
Glutamate metabolism
D-Gln & D-Glu metabolism

B. *Establishing a working metabolic map*

A way to simplify the reaction system is to confine the analysis to the major arteries of materials flows. In general, that will include glucose and amino acid metabolism biosynthesis of building blocks of biomass and products. On the biosynthesis, cells can only synthesize non-essential amino acids, not the essential ones. They also synthesize lipids, nucleotides and other cellular components. Since nearly all amino acids are supplied by medium in cell culture, the amino acid biosynthesis reactions can be neglected. The simplified reaction network thus includes only glucose and amino acid catabolism and synthesis of biomass and products.

Keep in mind that the simplification of reactions basically neglects all the other fluxes into other reactions.



A simplified metabolic network considering only carbohydrate, amino acid metabolism, cell and product syntheses.

C. *Establishing stoichiometric equations*

1. Central Metabolic Pathways:

- GLC → 2 PYR
- PYR → LAC
- PYR → AcCoA + CO_2
- AcCoA + OAA → αKG + CO_2
- αKG → SucCoA + CO_2
- SucCoA → FUM
- FUM → MAL
- MAL → OAA
- GLN → GLU + NH_3
- GLU → αKG + NH_3
- MAL → PYR + CO_2

2. **BIOMASS FORMATION:**

- 0.0208 GLC + 0.0377 GLN + 0.0133 ALA + 0.0070 ARG + 0.0 ASN + 0.0261 ASP+ 0.0004 CYS + 0.0006 GLU + 0.0165 GLY + 0.0033 HIS + 0.0084 ILE + 0.0133 LEU + 0.0101 LYS + 0.0033 MET + 0.0055 PHE + 0.0081 PRO + 0.0099 SER + 0.0080 THR + 0.0040 TYR + 0.0096 VAL → BIOMAS

3. **AMINO ACID METABOLISM:**

- PYR + GLU → ALA + αKG
- SER → PYR + NH_3
- 2 GLY → SER + CO_2 + NH_3
- CYS → PYR + NH_3
- ASP + αKG → OAA + GLU
- ASN → ASP + NH_3
- HIS → GLU + 2 NH_3 + CO_2
- ARG + αKG → 2 GLU + 2 NH_3 + CO_2
- PRO → GLU
- ILE + αKG → SucCoA + AcCoA + GLU
- VAL + αKG → GLU + CO_2 + SucCoA
- MET + SER + αKG → CYS + SucCoA + GLU
- THR → SucCoA + NH_3
- PHE → TYR
- TYR + αKG → GLU + FUM + 2 AcCoA + CO_2
- LYS + 2 αKG → 2 GLU + 2 CO_2 + 2 AcCoA
- LEU + αKG → GLU + 3 AcCoA

4. **ANITBODY PRODUCTION:**

- 0.0104 GLN + 0.0110 ALA + 0.0050 ARG + 0.0072 ASN + 0.0082 ASP + 0.005 CYS + 0.0107 GLU + 0.0145 GLY + 0.0035 HIS + 0.0050 ILE + 0.0142 LEU + 0.0145 LYS + 0.0028 MET + 0.0072 PHE + 0.0148 PRO + 0.0267 SER + 0.0160 THR + 0.0085 TYR + 0.0189 VAL → AB

AB: Antibody; **AcCoA**: Acetyl coenzyme A; **αKG**: α-ketoglutarate; **ALA**: Alanine; **ARG**: Arginine; **ASN**: Asparagine; **ASP**: Aspartate; **BIOMAS**: Biomass; **CYS**: Cysteine; **CO_2**: Carbon dioxide; **FUM**: Fumarate; **GLC**: Glucose; **GLN**: Glutamine; **GLU**: Glutamate; **GLY**: Glycine; **HIS:** Histidine; **ILE**: Isoleucine; **LAC**: Lactate; **LEU**: Leucine; **LYS**: Lysine; **MAL**: Malate; **MET**: Methionine; **NH_3**: Ammonia; **OAA**: Oxaloacetate; **PHE**: Phenylalanine; **PRO**: Proline; **PYR**: Pyruvate; **SER**: Serine; **SucCoA**: Succinate coenzyme A; **THR**: Threonine; **TYR**: Tyrosine; **VAL**: Valine

D. Selecting elements for balances

Example: Balance on C and N (H and O are difficult to account for). All terms and reactions not involving carbon and nitrogen derived from externally supplied nutrients are dropped.

1. **GLYCOLYSIS:**
   a) GLC - 2 PYR = 0
   b) PYR - LAC = 0
2. **TCA CYCLE**
   a) PYR - AcCoA - CO2 = 0
   b) AcCoA + OAA - αKG - CO2 = 0
   c) αKG - SucCOA - CO2 = 0
   d) SucCoA - FUM = 0
   e) FUM – MAL = 0
   f) MAL - OAA = 0
3. **GLUTAMINOLYSIS:**
   a) GLN - GLU - NH3 = 0
   b) GLU - αKG - NH3 = 0
   c) MAL - PYR - CO2 = 0
4. **BIOMASS SYNTHESIS AND PRODUCT: (EXAMPLE)**
   a) 0.0208 GLC + 0.0377 GLN + 0.0133 ALA + 0.0070 ARG + 0.0 ASN + 0.0261 ASP + 0.0004 CYS + 0.0006 GLU + 0.0165 GLY + 0.0033 HIS + 0.0084 ILE + 0.0133 LEU + 0.0101 LYS + 0.0033 MET + 0.0055 PHE + 0.0081 PRO + 0.0099 SER + 0.0080 THR + 0.0040 TYR + 0.0096 VAL - $CH_{1.975}N_{0.2605}O_{0.489}$ = 0
   b) 0.0104 GLN + 0.0110 ALA + 0.0050 ARG + 0.0072 ASN + 0.0082 ASP + 0.005 CYS + 0.0107 GLU + 0.0145 GLY + 0.0035 HIS + 0.0050 ILE + 0.0142 LEU + 0.0145 LYS + 0.0028 MET + 0.0072 PHE + 0.0148 PRO + 0.0267 SER + 0.0160 THR + 0.0085 TYR + 0.0189 VAL - $CH_{1.540}N_{0.2645}O_{0.3146}$ = 0
5. **AMINO ACID METABOLISM**
   a) PYR + GLU - ALA – AKG = 0
   b) SER - PYR - NH_3 = 0
   c) 2GLY - SER - CO_2 - NH_3 = 0

d) CYS - PYR - NH_3 = 0
e) ASP + AKG - OAA - GLU = 0
f) ASN - ASP - NH_3 = 0
g) HIS - GLU - $2NH_3$ - CO_2 = 0
h) ARG + αKG - 2 GLU - $2NH_3$ - CO_2 = 0
i) PRO - GLU = 0
j) ILE + αKG - SucCoA - AcCoA - GLU = 0
k) VAL + αKG - GLU - CO2 - SucCoA = 0
l) MET - SER + αKG - CYS - GLU - SucCoA = 0*
m) THR - SucCoA - NH3 = 0
n) PHE - TYR = 0
o) TYR + αKG - GLU - FUM - 2 AcCoA - CO_2 = 0
p) LYS + 2 αKG - 2 GLU - 2 CO2 - 2 AcCoA = 0
q) LEU + αKGα - GLU - 3 AcCoA = 0

*A methyl group transfer reaction is neglected. This simplification does not make a significant difference in flux calculation, as the flux of this reaction is usually very small.

E. Setting up material balance equations

These reaction equations can be written in spreadsheet format to line up each compound, with reactants being negative and products having positive values. The next step is to obtain the stoichiometric coefficient matrix (A). The vector (x) consists of the rate of each reaction (which is given names of Jxxx-xxx). The product vector (r) represents the net rate of each compound from all reactions in which it is involved. For example, Acetyl CoA is involved in six reactions, five as product and one as reactant, the first element of the vector r is the sum of all the fluxes of Acetyl CoA ($J_{PYR\text{-}ACC}$, $J_{OAA\text{-}\alpha KG}$, $J_{ILE\text{-}SUC}$, $J_{TYR\text{-}FUM}$, $J_{LYS\text{-}ACC}$, and $J_{LEU\text{-}ACC}$).

We next assume that the concentrations of all the compounds inside the cells are at steady state values. Under such conditions, the net rate (or rate of accumulation) of all intracellular compounds is 0. Therefore, the value of all the elements corresponding to metabolic intermediates in vector r is 0. Since cells are taking up nutrients, the values of elements corresponding to nutrients will be their specific consumption or production rate. The value for biomass (product) will be its specific growth rate.

See the Excel file for setting up the equations.

F. Obtaining experimental specific rates

See Excel template for details.

G. Biomass equation

Biomass equation is one of the most contentious issues in MFA. One needs to know cell's elemental composition in order to set up an equation for biomass synthesis. It is very difficult to determine what materials go directly to biomass. In the above example, biosynthesis of amino acids, fatty acids and other lipids are not considered. Fortunately, under most cultivation conditions, biomass is only a small portion of output as compared to the metabolites.

In general, there are two different approaches to developing the biomass equation, and both require some knowledge of cellular composition in terms of C, N, O, H or fraction of lipids, proteins, nucleic acids, polysaccharides. In the more detailed approach, the biosynthetic reactions to form lipids, protein, nucleic acids (DNA, RNA) and other cellular components from various nutrients are established using known pathways. These equations are then combined to give the biomass equation. In the second and more simplified approach, the amino acids composition of proteins and those required to synthesize nucleic acids are used to develop the stoichiometric coefficients for various amino acids in the biomass equation. The other coefficients for glucose are derived by balancing carbon in the equation; if necessary, glutamine is assumed to provide extra nitrogen to provide N balance.

1. A GENERAL APPROACH TO DEVELOP A BIOMASS EQUATION

The biomass equation is expressed as a lumped equation combining the major reactions for synthesizing cellular building blocks. As a precursor to biomass, glucose is required for the synthesis of lipids, ribose and deoxyribose in nucleotides. Amino acids are required for the synthesis of cellular proteins and the product, IgG. A number of amino acids are also used in the synthesis of nucleotides. All these metabolic events are lumped into the biomass equation.

A general chemical reaction representing the carbon and nitrogen balance for biomass formulation from glucose, glutamine and other amino acids is written as

$$a\ C_6H_{12}O_6 + b\ C_5H_{10}N_2O_3 + \sum c_i\ C_{wi}H_{xi}N_{yi}O_{zi} \rightarrow\ CH_\alpha N_\beta O_\gamma \qquad \text{(Eq. 1)}$$

where $C_6H_{12}O_6$, $C_5H_{10}N_2O_3$, and $CH_\alpha N_\beta O_\gamma$ are the chemical formulae for glucose, glutamine and cell mass, respectively. $c_i\ C_{wi}H_{xi}N_{yi}O_{zi}$ denotes the contribution from each amino acid to the biomass synthesis. The stoichiometric coefficient, c_i, for each amino acid is then the sum of their contributions to cellular proteins and nucleotides. In our analysis we dealt with the balances of C and N only.

The elemental composition of the cell was determined to be $CH_{1.975}N_{0.2605}O_{0.489}$ (elemental analysis done by MHW Labs, Phoenix, AZ). The cellular molecular weight was calculated as

25.474 g/mol from the elemental composition of the cell. The cell composition of biomass is assumed to be: 75% protein; 7.5% RNA; 3.75% DNA; and 13.75% lipids and other macromolecules (Darnell et al., 1986). The amino acid composition of representative mammalian cells (Okayasu et al., 1997) was used as the amounts of each individual amino acid needed for synthesizing the cellular protein (Table I).

Five amino acids, glutamine, glutamate, aspartate, serine and glycine, are involved in nucleic acid biosynthesis. Based on the nucleic acid biosynthetic pathways, the following stoichiometric equations for synthesizing purine and pyrimidine are derived:

$$0.5GLC + 2GLN + 2ASP + GLY \rightarrow C_5N_5H_4 \text{ (Adenine)} + 2GLU + 2FUM \quad \text{(Eq. 2)}$$

$$0.5GLC + 3GLN + ASP + GLY \rightarrow C_5N_5H_4O \text{ (Guanine)} + 3GLU + FUM \quad \text{(Eq. 3)}$$

$$2GLN + ASP \rightarrow C_4N_3H_4O \text{ (Cytosine)} + 2GLU \quad \text{(Eq. 4)}$$

$$SER + ASP + GLN \rightarrow C_5N_2H_5O_2 \text{ (Thymine)} + GLU + GLY \quad \text{(Eq. 5)}$$

$$ASP + GLN \rightarrow C_4N_2H_3O_2 \text{ (Urasil)} + GLU \quad \text{(Eq. 6)}$$

Assuming that purine and pyrimidine are present in equal molar amount in the cell, the molar contribution of purine and pyrimidine for biomass synthesis can be estimated from the DNA and RNA content (3.75% and 7.5% of biomass by mass respectively) to be 0.009 mol/mol cells. The stoichiometric coefficient for each amino acid in the cell mass formation equation (excluding glutamine, glutamate, aspartate, serine and glycine) can then be calculated from its contribution to cellular protein. Those for glutamate, aspartate, serine and glycine are then their contribution to both cellular protein and nucleotides (Table I).

The stoichoimetric coefficients, a and b, for glucose and glutamine are determined by closing carbon and nitrogen balances from Eq.1. The resulting cell equation is shown in II.C. The equation for antibody production is based on the amino acid composition of a typical IgG molecule (Edelman et al., 1969).

Table I. Estimation of the coefficients of glucose and amino acids for the biomass equation.

	Occurrence in cellular protein		Occurrence in cellular DNA/RNA	Stoichiometric coefficient for biomass eqn
	% mol	mol AA/mol cell	mol AA/mol cell	
ALA	9.03	0.0133		0.0133
ARG	4.74	0.0700		0.0070
ASP	10.08	0.0149	0.0113	0.0261
CYS	0.26	0.0004		0.0004
GLU	12.62	0.0186	-0.0180	0.0006
GLY	9.14	0.0135	0.0030	0.0165
HIS	2.22	0.0033		0.0033
ILE	5.73	0.0084		0.0084
LEU	9.00	0.0133		0.0133
LYS	6.85	0.0101		0.0101
MET	2.27	0.0033		0.0033
PHE	3.73	0.0055		0.0055
PRO	5.51	0.0081		0.0081
SER	6.19	0.0091	0.0008	0.0099
THR	5.42	0.0080		0.0080
TYR	2.73	0.0040		0.0040
VAL	6.54	0.0096		0.0096
GLN		0.0197	0.0180	0.0377
GLC		0.0208		0.0208

H. Sensitivity analysis

Determine which measurement has the most profound effect on the calculated fluxes. A large error in the measurements with a large value of sensitivity coefficient will have a major effect on the outcome of analysis. Therefore, caution needs to be taken to ensure accuracy of these measurements.

I. Solution and flux visualization

MFA is a powerful tool in comparing internal fluxes under different culture conditions or growth stages. However, the results of MFA involve tens of variables depicting the fluxes in the reaction network and are difficult to comprehend without a proper graphic presentation. Linking the results of analysis to a metabolic map provides an excellent way of viewing the fluxes.

In the template included in the CD-ROM, the experimental data of specific rates is entered into an Excel spreadsheet to check for material balances and transferred to the MFA program for calculation. The obtained flux results are transferred back to the Excel spreadsheet, and presented as charts on a metabolic map which is displayed in a worksheet. Furthermore, the charts and metabolic map are also dynamically linked to a metabolic chart in a PowerPoint file for presentation. In this way, the results of MFA can be viewed easily in a graphic form of the metabolic map and be readily available for group discussion.

III. BRIEF SUMMARY

MFA is an important analysis technique of quantitative physiology and metabolic engineering. A flux balance can be written for each metabolite (y_i) within a metabolic system to yield the dynamic mass balance equations that interconnect the various metabolites. Generally, for a metabolic network that contains *m* compounds and *n* reactions, all the transient material balances can be represented by a single matrix equation:

$$dY/dt = A\,X(t) - r(t)$$

where

Y: m dimensional vector of intracellular concentrations of intermediates, metabolites and nutrients

X: n metabolic fluxes

A: Stoichiometric *m* x *n* matrix, and

r: vector of *m* specific rates

The time constants characterizing metabolic transients are typically very rapid compared to the time constants of cell growth and process dynamics, therefore, the mass balances can be simplified to only consider the steady-state behavior. Eliminating the derivative yields

$$A\,X(t) = r(t)$$

Provided that $m >= n$ and *A* is full rank, the weighted least squares solution of the above equation is:

$$X = (A^T A)^{-1} A^T r$$

The sensitivity of the solution can be investigated by the matrix

$$dX/dr = (A^T A)^{-1} A^T$$

The elements of the above matrix are useful for the determination of the change of individual fluxes with respect to the error or perturbation in the measurements.

A salient feature of this integrated approach is the streamlined data manipulation and final visualization of the results. MFA is a powerful tool, but its execution can also be time consuming. By streamlining the process, we can make the process become a routine, reveal more information from the data. One should be reminded that MFA is a tool based on many assumptions. It is best used for a first approximation to obtain some insights for further exploration of ideas.

Return to the Table of Contents