METSTOICH

Teaching Quantitative Metabolism and Energetics in Biochemical Engineering

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Biochemistry is one of the important foundation courses in a biochemical engineering curriculum. It provides a basic introduction of cellular metabolism to engineering students to teach them how raw materials can be converted into valuable metabolic products by microorganisms in various bioprocesses. Teaching metabolism in biochemical engineering courses normally adopts the traditional "biochemistry approach." Students are presented with a number of reaction pathways that make major cell components (*e.g.*, protein, RNA, DNA, lipids, cell walls) as well as major catabolic products using a qualitative description. Traditional chemical engineering courses, however, focus on product yield, selectivity, reaction rate, and reactor/process design. It is similar for biochemical engineers that product yield, biomass yield, and ATP yield are important parameters for bioreactor design. All these goals, if applied to a biochemical system, require a quantitative knowledge of metabolism. Therefore, a quantitative description in metabolism can complement the major skill base of engineering students and is more consistent with the overall philosophy or learning outcomes of an engineering degree.

As a subset of system biology, metabolic engineering focuses on the metabolism of one organism. It is the practice of purposeful modification of metabolism using recombinant DNA technology along with mathematical analysis to optimize genetic and regulatory processes within the cell. This leads to the modification of the cell's properties to achieve a desirable objective.[1-4] Metabolic Flux Analysis (MFA, also known as metabolite balancing, metabolic flux balancing, etc.), is a practical tool for understanding and analyzing metabolic pathways, pathway interaction, and control. Varma and Palsson[5] suggested that there are five major applications for MFA, namely: 1) to quantify metabolic physiology, 2) to simulate and interpret experimental data, 3) to analyze metabolic pathways for metabolic engineering, 4) to optimize cell culture medium, and 5) to design and optimize bioprocesses. MFA is an analytical tool developed based on stoichiometric network models,[6] and it is assumed that those metabolic fluxes are in steady state when compared with growth and other processes. Unlike simulations based on mechanistic models that require detailed enzyme kinetic data, MFA is used to analyze the metabolic flux map and only requires metabolic reaction pathway details and stoichiometry, growth metabolism, and several strain-specific parameters. MFA determines a domain of stoichiometrically allowable flux distributions.[5] Even if several restrictions are enforced, for complete metabolite balancing of a cell, a very large amount of flux data needs to be analyzed to accurately represent the interactions between the various metabolic pathways. Practically, such analysis is assisted by specifically designed software packages that simulate the metabolic networks.

The analysis provided by MFA is also good for demonstration of the quantitative aspects of metabolism to students. Most analytical software packages, however, are developed for research purpose and mainly focus on pathway control (*i.e.*, metabolic control analysis, MCA). Metstoich was initially developed to focus on teaching metabolism and to link practical biochemical engineering parameters with metabolic flux analysis.

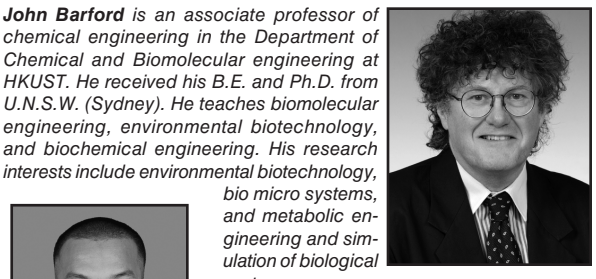

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TABLE 1
Parameters as Input and Output for Various Calculation Modes in Metstoich

Parameters	Problem Types			
	(a) Theoretical Y_{XS}	(b) Experimental Y_{XS}	(c) Predefined $Y_{X/ATP}$	(d) Experimental Y_{XS} with Predefined $Y_{X/ATP}$
(1) Cell compositions	Input	Input	Input	Input
(2) Glucose usage for energy generation process	Input	Input	Input	Input
(3) P/O ratio	Input	Input	Output	Output
(4) ATP efficiency	Input	Output	Input	Output
(5) Y_{XS}	Output	Input	Output	Input
(6) $Y_{X/ATP}$	Output	Output	Input	Input

EXISTING SOFTWARE PACKAGES

To explore the physiological properties of biological systems, a system of equations must be solved. Such a task can be easily done with the aid of modern personal computers and metabolic engineering software packages. Some important and/or widely used software packages are:

GEPASI 3[7-8] is a widely used free biochemical reactions simulation software package. GEPASI simulates the kinetics of biochemical reaction systems and provides functions such as metabolic control analysis (MCA), elementary mode analysis (EMA), optimization, and parameter fitting. The last version of GEPASI released was 3.30 in September 2002. COPASI[9] was developed based on GEPASI with different simulation techniques, optimization routine, etc. Jarnac (a.k.a. Scamp II)[10] simulates the steady state and transient behavior of metabolic pathways and calculates all coefficients for MCA.

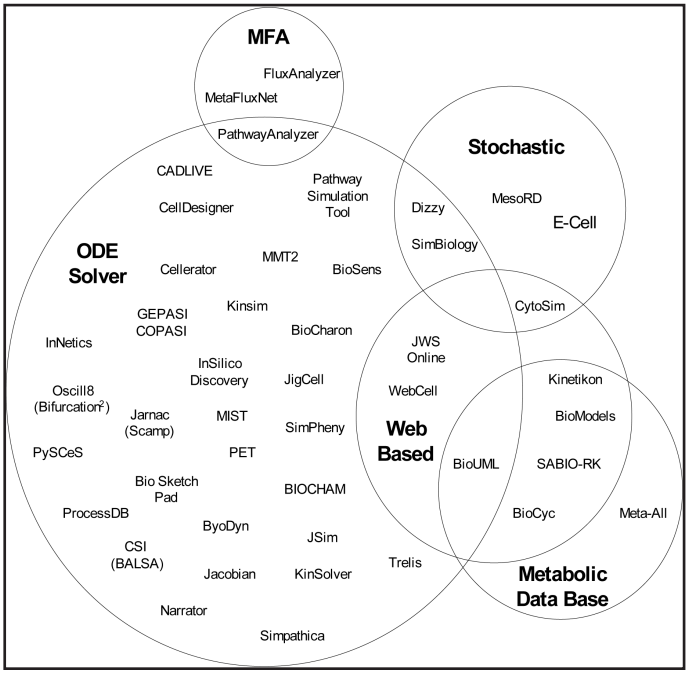


Figure 1 (left). *Some existing metabolic engineering software packages/projects.*

E-Cell[11] is an object-oriented, whole-cell simulation software package. MIST[12] performs dynamic simulations, stoichiometric calculations, and MCA. JWS Online[13] is an Internet-based metabolic simulator with collections of several metabolic models, and it can provide MCA to analyze the simulation results. KINSIM[14,15] is a rate equation-based numerical simulator and it was used for the simulation of enzymatic reaction system kinetics. FluxAnalyzer[16] is a MATLAB package with GUI for stoichiometric analysis of metabolic networks. It can provide functions such as MFA, flux optimization, topological features detection, and pathway analysis. In one of the more extensive examples, Klamt, et al.,[17] carried out a metabolic flux analysis on Purple Nonsulfur Bacteria by using FluxAnalyzer. This model involved 30 of the most important catabolic branchpoint-metabolites (intermediate metabolites to which at least three reactions are linked) and 41 catabolic reactions—1 for growth rate, 25 for central metabolic pathways, and 7 for photosynthesis, cyclic electron transport during photosynthesis, respiration, ATP synthesis, and maintenance. The model also involved 46 anabolic reactions using the stoichiometries presented in Neidhardt, et al.[18]

Except for FluxAnalyzer, all above simulation packages focus on the dynamic behavior of metabolic pathways. They require reaction kinetics as input and some of them can even perform metabolic pathway analysis such as MCA.

Other than the above-listed software packages, there are many packages/projects developed or under development. Figure 1 summarizes part of the metabolic engineering software packages/projects found. Most of them are ODE solvers, some of which can perform sensitivity analysis (MCA). Some, however, were developed for various purposes, such as:

- *CellDesigner[19] is for gene-regulatory and biochemical networks.*
- *Cellerator[20] is a Mathematica package designed for modeling with automated equation generation. It was designed with the intent of simulating signal transduction.*
- *InNetics[21] was developed for genomic-based drug discovery.*
- *The JigCell project[22] explores the cell physiology from the scope of molecular regulatory networks.*

There are two trends for metabolic software development.

The first trend is using visual tools to allow users to construct pathway models. The second is the development of Web-based applications. Nowadays, the Internet is already part of daily life and Web-based applications are a good choice, especially for database projects to collect and share data.

The common advantage of these packages is that you can input any model to the package for analysis. Their practical use for engineering purposes, however, is limited and is not their primary purpose. They do not address issues of energetics and ATP usage, the production of biomass yield, etc.

METSTOICH

Metstoich was initially developed for teaching purposes[23-24] and is based on the metabolism of a specific yeast, *S. cerevisiae*.[25] Metstoich includes the following major pathways: 1) central metabolic pathways, such as glycolysis, tricarboxylic acid (TCA) cycle and pentose-phosphate pathway (PPP); and 2) biosynthetic pathways. The central metabolic pathways serve to provide precursors for biosynthetic pathways, and for generating energy (ATP) to support cell growth and maintenance.

The main purpose of Metstoich is to link metabolic flux distribution among pathways with practical engineering parameters encountered in a standard biochemical engineering course, such as biomass yield (Y_{XS}), product yield (Y_{PS}), ATP yield ($Y_{X/ATP}$), etc. Pathway reactions are predefined and based on a specific yeast. Such an approach could also help to identify flux distribution among branch points.

There are several important inputs necessary for Metstoich to determine the flux map:

1) *Cell macromolecular composition;*
2) *Glucose distribution (usage) in central metabolic pathways for energy generation process;*
3) *P/O ratio;*
4) *ATP utilization efficiency (or simply called as ATP efficiency, η), the percentage of total ATP generated that is directly consumed in biosynthetic reactions;*
5) *Biomass yield, Y_{XS};*
6) *ATP yield, $Y_{X/ATP}$.*

There are four problem types that can be solved by Metstoich with above inputs:

a) *Calculation based on theoretical yield; or*
b) *Calculation based on experimental biomass yield, Y_{XS}; or*
c) *Calculation based on predefined ATP yield, $Y_{X/ATP}$; or*
d) *Calculation based on experimental biomass yield, Y_{XS}, and predefined ATP yield, $Y_{X/ATP}$.*

Table 1 summarizes a matrix of problem types, inputs, and outputs, and Figure 2 shows part of the input interface.

Users can specify: the cell composition (Figure 2); carbon source and electron donor if CO_2 is the carbon source (Figure 3); electron

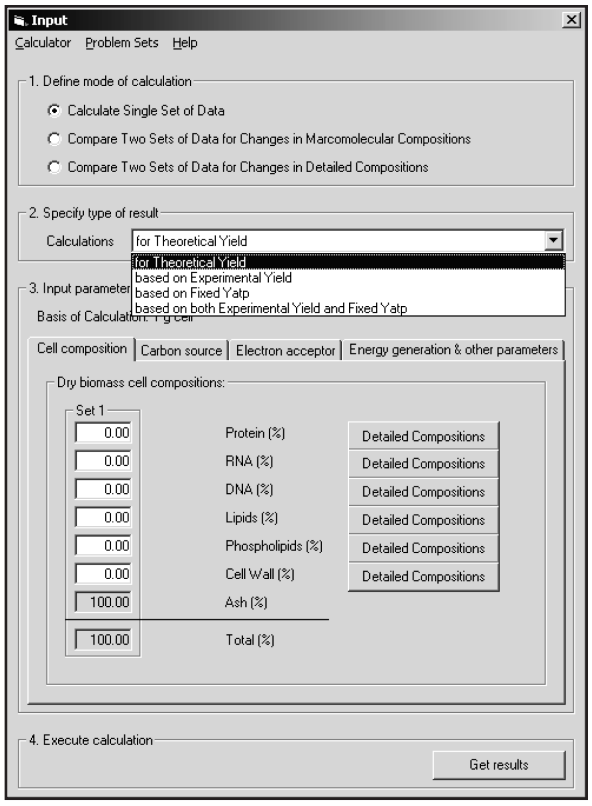


Figure 2. *Part of Metstoich input interface—basic information and cell compositions.*

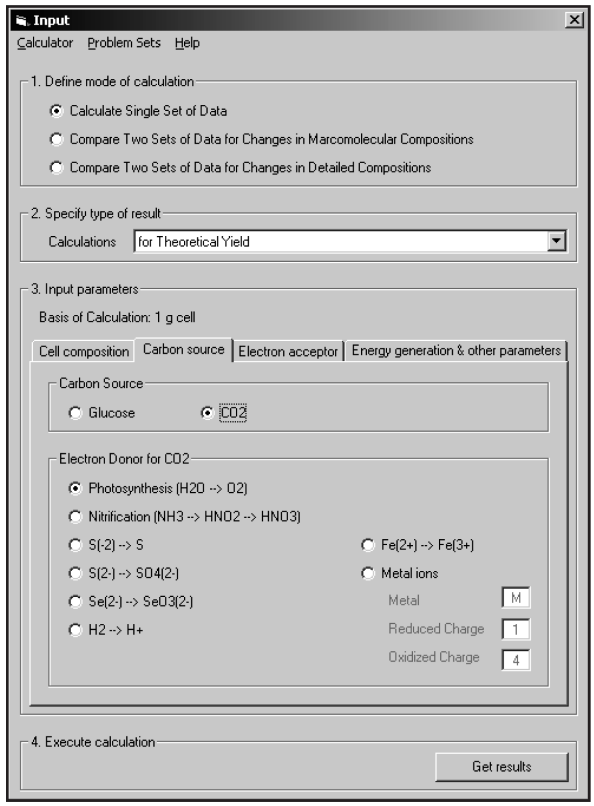
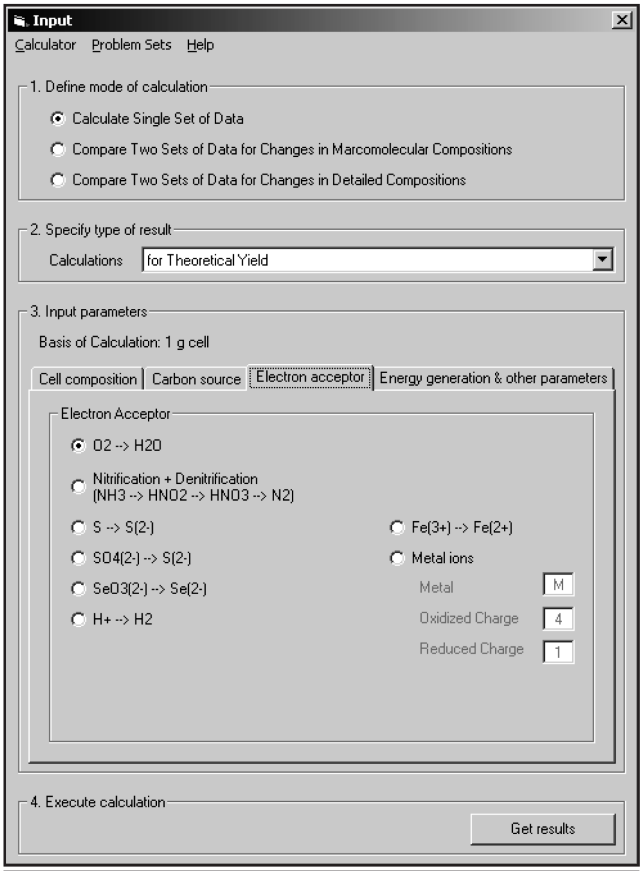
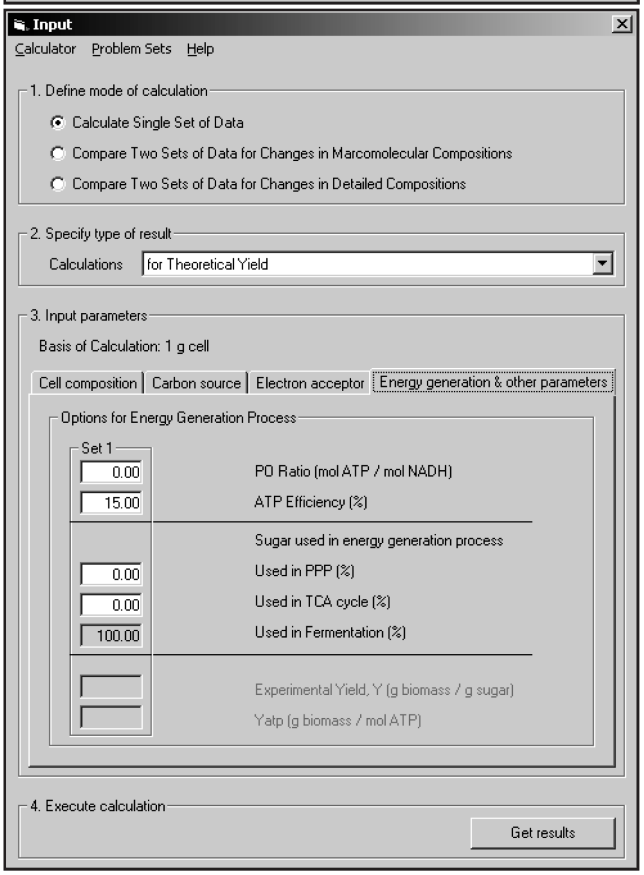


Figure 3. *Part of Metstoich input interface—carbon source.*





acceptor (Figure 4); and energetic issues of the microorganism (Figure 5). Metstoich can compare two sets of metabolic flux maps (Figure 6) and highlights fluxes with a defined degree of difference in percentage.

The results generated by Metstoich are organized into several levels of detailed worksheets with biochemical detail and illustrative reaction pathways included to make it more understandable. Levels of organized results are:

Cell Yield and Energetics *(Figure 7) – This worksheet is the executive summary of the overall performance of the cell with the inputted common engineering parameters;*

Fate of Glucose *(Figure 8) – This worksheet summarizes how much glucose is used for specified purposes via specified pathways;*

Figure 4. (top left) *Part of Metstoich input interface —electron acceptors.*

Figure 5. (bottom left) *Part of Metstoich input interface —energetic and other parameters.*

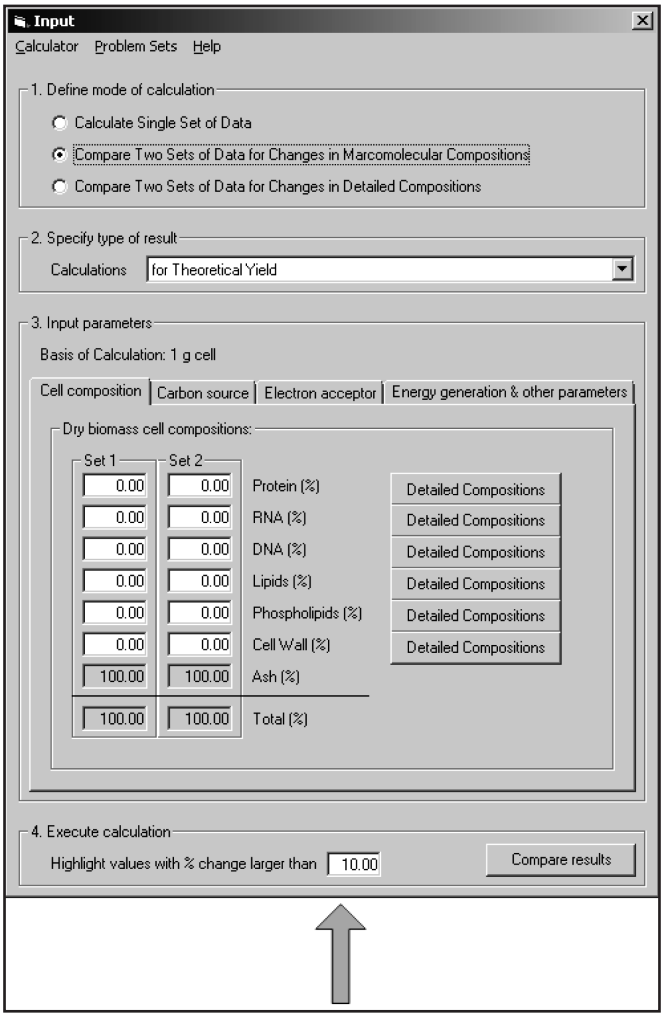


Figure 6. (above) *User can specify highlight values that changed larger than the given percentage.*

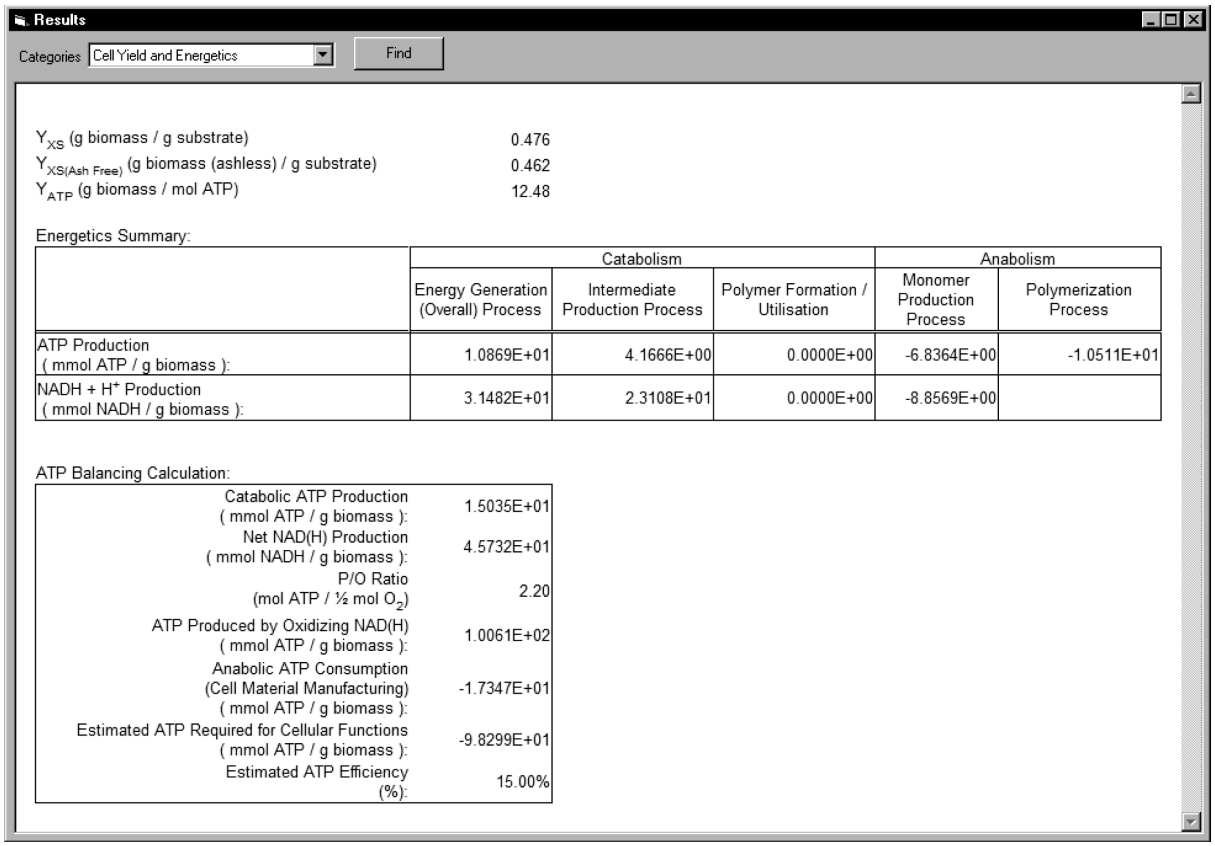


Figure 7. *"Cell Yield and Energetics," the cell yield (either estimated or given), $Y_{X/ATP}$, amount of ATP generated directly from reactions or oxidative phosphorylation are summarized.*

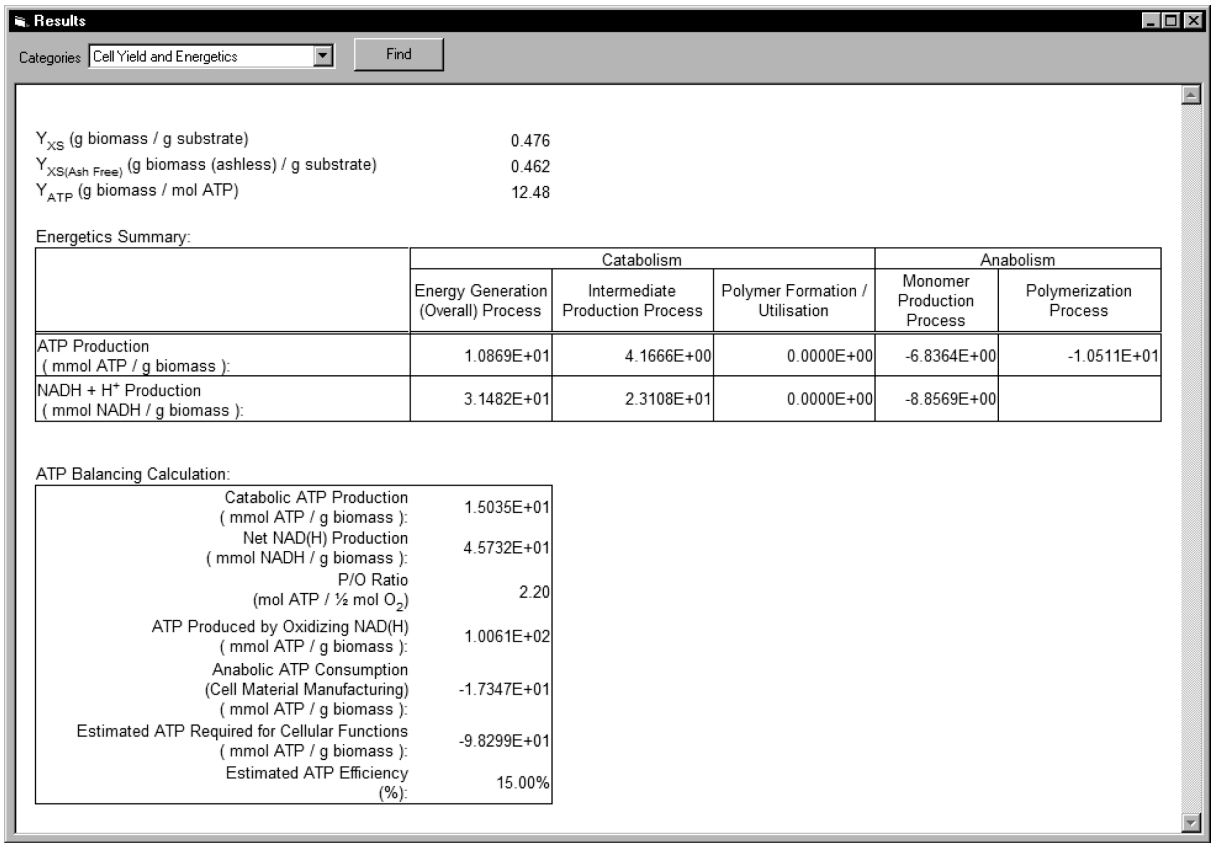


Figure 8. *"Fate of Glucose," glucose directly linked with biosynthesis or energy generation process is analyzed.*

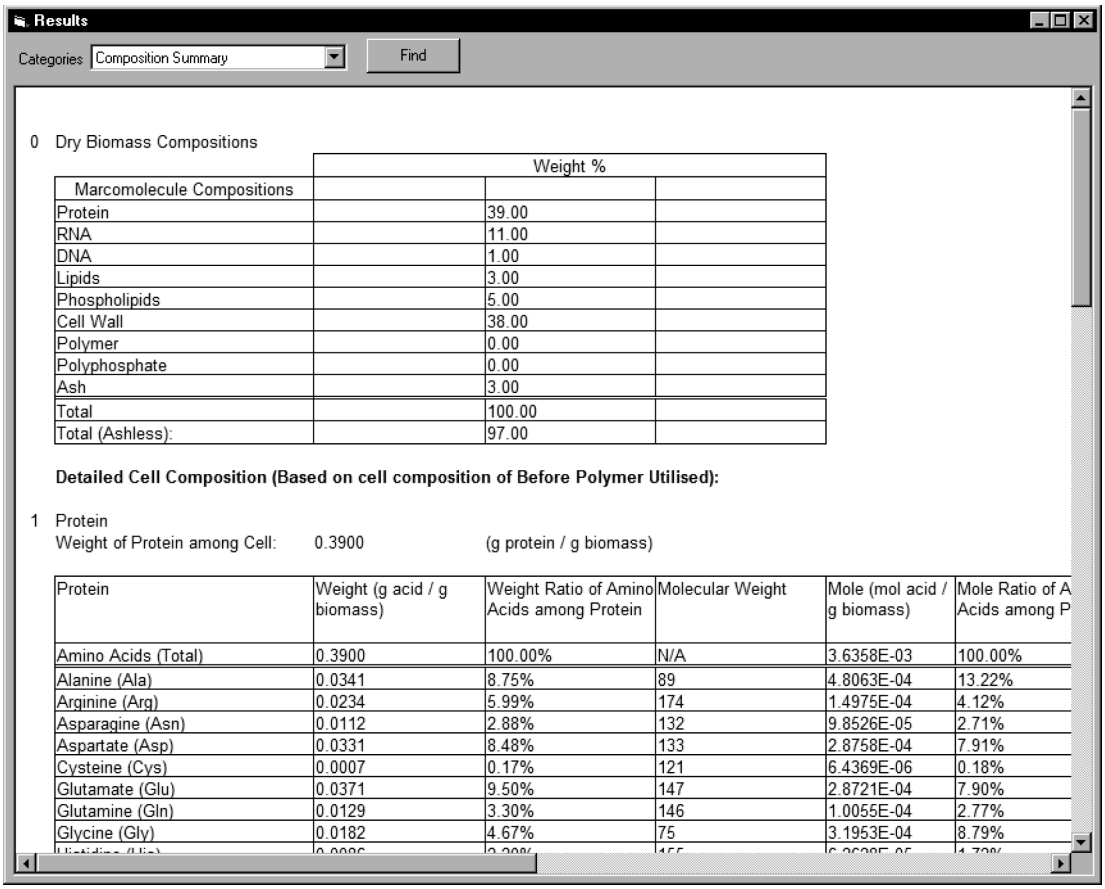


Figure 9. *All detailed biomass compositions, such as amino acid, etc., are summarized in "composition summary."*

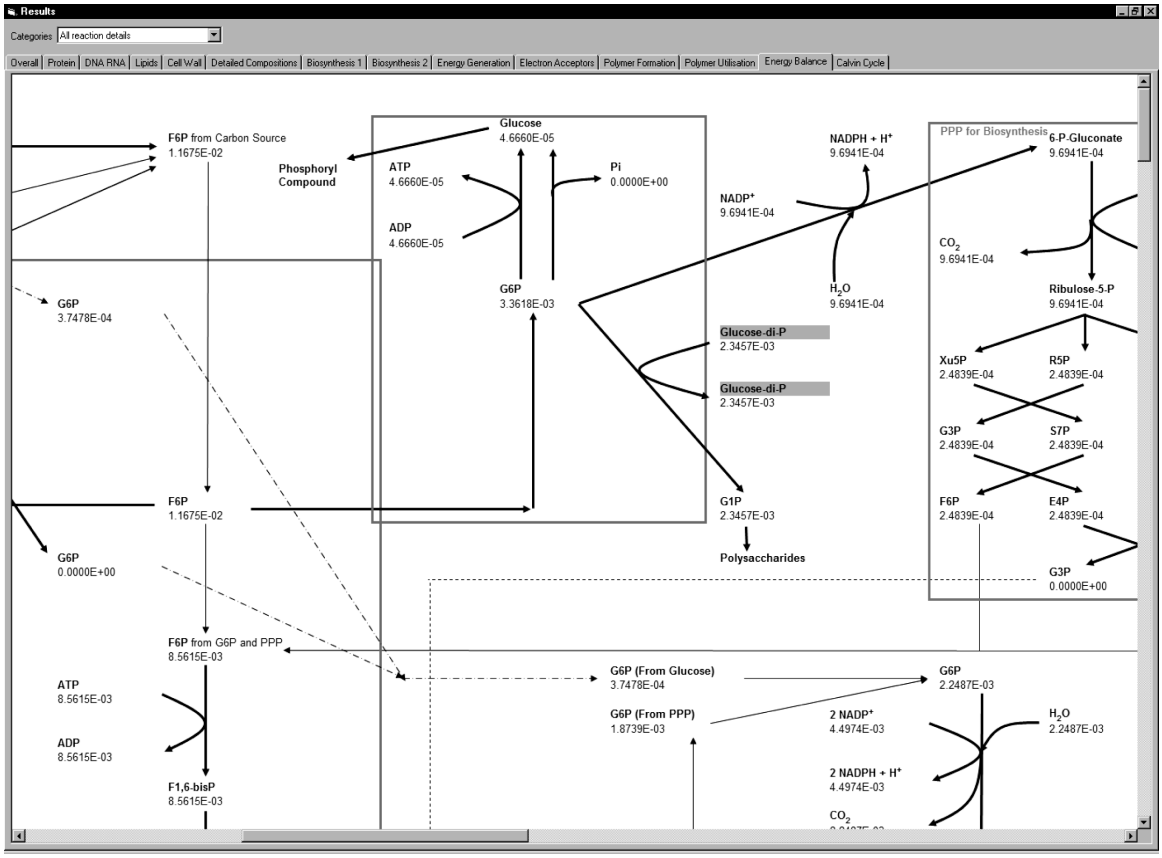


Figure 10. *"All Detailed Reactions" shows all biochemical reactions.*

Composition Summary *(Figure 9) — This worksheet summarizes cell compositions and their detail; and*

All Detailed Reactions *(Figure 10) – This worksheet shows the detailed flux maps for biosynthetic pathways—central metabolic pathways used for either biosynthesis purpose or energy generation purposes.*

Metstoich already contains amino acid production pathways and it is capable of analyzing amino acid production. Since Metstoich already contains information on major catabolic and anabolic pathways, it is easy to further include more production formation pathways such as antibodies, biofuel, etc.

Metstoich is focused on the static metabolic flux analysis, and therefore enzyme concentrations, kinetic expressions, intermediate concentrations, and thermodynamics have not been incorporated. An extension of Metstoich that incorporates thermodynamics and reaction kinetics, etc., has been developed and reported.[26-28]

The core calculation module of Metstoich is written using Microsoft Excel 2002 with VBA Macro. This core Excel module is responsible for constructing and displaying the metabolic flux map. The front-end graphical user interface was written in Visual Basic. Metstoich runs on Microsoft Windows 98, 2000, XP, and Vista with Microsoft Office 2000, XP, or 2003 installed.

Example to Demonstrate the Teaching of Quantitative Metabolism to Students

This is an example problem that students undertake as an exercise. It is taken from a number of problems included in the Metstoich package:

> The biomass composition (weight %) of a given yeast is as follows:
>
> Protein = 39%, DNA = 1%, RNA = 11%, Lipids = 3%, Phospholipids = 5%, Cell Wall = 38%, and Ash = 3%
>
> For energy generation, 10% glucose is used by pentose phosphate pathway, 60% glucose is used by the TCA cycle and 30% glucose is used by the fermentation pathway. The reported biomass yield is 0.4 g-biomass / g-glucose and let P/O ratio be 2.2 mol-ATP / mol-NADH. What is the corresponding $Y_{X/ATP}$ and ATP efficiency. What is the relationship between P/O ratio and $Y_{X/ATP}$?

Since Y_{XS} with P/O ratio are given, the "Experimental Y_{XS}" calculation mode should be used. With given input values, Metstoich returns $Y_{X/ATP}$ = 7.85 g-biomass / mol-ATP and ATP efficiency is 10.6%. And the relationship between $Y_{X/ATP}$ and P/O ratio is shown in Figure 11 at various P/O ratios:

With fixed Y_{XS} and cell compositions, glucose directly consumed to form biomass is always fixed at 1.43 g-glucose / g-biomass. The total glucose consumed is 2.5 g-glucose / g-biomass for the given Y_{XS} = 0.4. Therefore glucose consumed to generate energy is always 1.07 g-glucose / g-biomass, and it always generates 17.3 mmol-ATP and 50.1 mmol-NADH per 1.07 g-glucose consumed in assigned pathways. Therefore, total ATP generated in energy generation process = (17.3 +

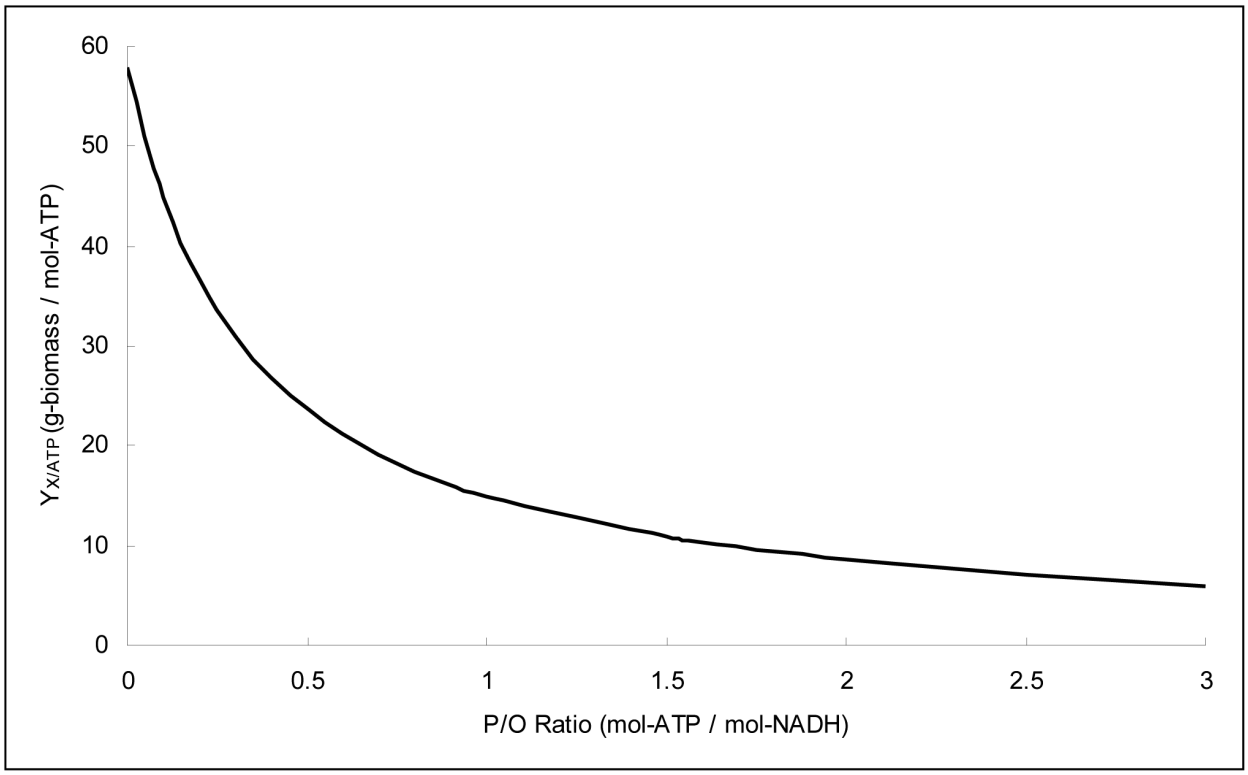


Figure 11. *Relationship between $Y_{X/ATP}$ and P/O ratio.*

P/O x 50.1 mmol-ATP) / 1.07 g-glucose. And $Y_{X/ATP}$ = 1 g-biomass / total ATP generated in energy generation process. It is suggested that normal $Y_{X/ATP}$ is around 10.5 g-biomass / mol-ATP. Using the "Experimental Y_{XS} and Fixed $Y_{X/ATP}$" calculation mode, it is found that the P/O ratio = 1.56 mol-ATP / mol-NADH and ATP efficiency = 14.27%.

Based on the fluxes given by Metstoich, students can draw simplified flux map as illustrated in Figure 12, Figure 13, and Figure 14 to understand the quantitative use of glucose by the cell and how much energy had been generated. By combining Figure 13 and Figure 14, students can generate an overall quantitative flux distribution for the given biomass.

COMMENTS ON METSTOICH

Professional evaluation was undertaken by Learnet of Hong Kong University. Metstoich had been reviewed by four leading academics in biochemical engineering from the U.K., the United States, Australia, and Singapore: Prof. D. Bogle from University College London, Prof. L. Nielsen from University of Queensland, Prof. D. Trau from National University of Singapore, and Prof. P. Fu from the University of Hawaii at Manoa. It was considered an excellent tool for learning of major biochemical engineering concepts such as $Y_{X/ATP}$, yield, etc. The feature that compared two sets of metabolic flux maps with a percentage change larger than a specified number was also highly regarded (Figure 6). In general, Metstoich has been rated as four stars out of five by these academics for different aspects such as interface design, quality of content, and learning potential.

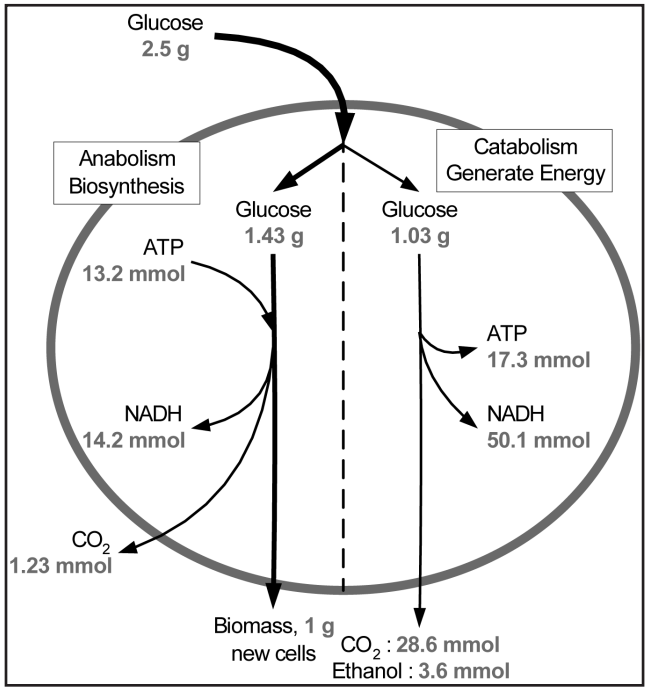


Figure 12. *Glucose used for biosynthesis and energy generation purposes, drawn based on Metstoich results.*

METSTOICH AS TEACHING TOOL

Metstoich has been applied in biochemical engineering and biochemistry classes at HKUST and it has been rated as easy to use by students. Students have been interviewed by the Center of Enhanced Learning & Teaching (CELT) of the Hong Kong University of Science and Technology (HKUST). It is agreed that Metstoich is easy to use, since the help functions and labels and buttons of the software are clear. The advantage of Metstoich is it can compare two sets of calculated results by highlighting the difference. Students felt that Metstoich contained too much information, however, since it covers from networks of reactions to energetics and cell yield, etc.

CONCLUSION

Engineering students are accustomed to quantitative concepts from their foundation courses. Biochemistry can also be taught quantitatively and when this is done, engineering students can appreciate the importance of metabolism in understanding and optimizing bioprocesses. Metstoich, a metabolic calculator for teaching purposes, was developed to introduce metabolism to students using quantitative principles. As such, it is useful to both engineering students and biochemistry/life sciences students, who normally do not have strong backgrounds or training in quantitative methods.

Metstoich has many novel features:

1. *Linking practical engineering parameters with cell growth, product yield, energetics, etc.;*
2. *Analyzing the flux though any reaction pathway;*
3. *Calculating how many nutrients are required for cell growth.*

Such analysis can provide useful information about how product yield is related with biomass yield, cell energetics, etc. Students can explore different metabolic options and are challenged to further explore their relationship to bioreactor/medium design.

The package has been well received by both academic experts in biochemical engineering and undergraduate chemical engineering and biochemistry students at HKUST.

ACKNOWLEDGMENT

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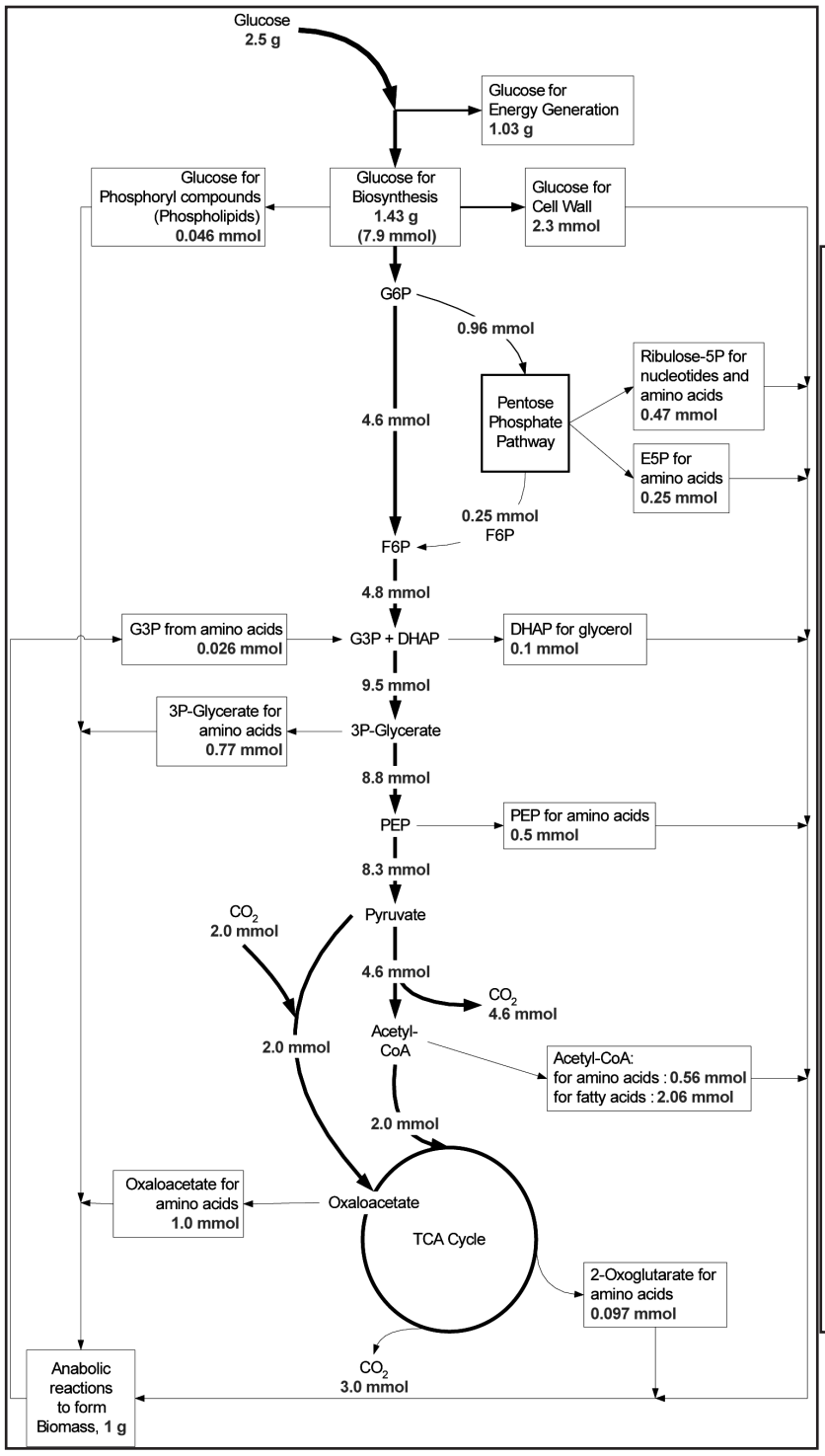
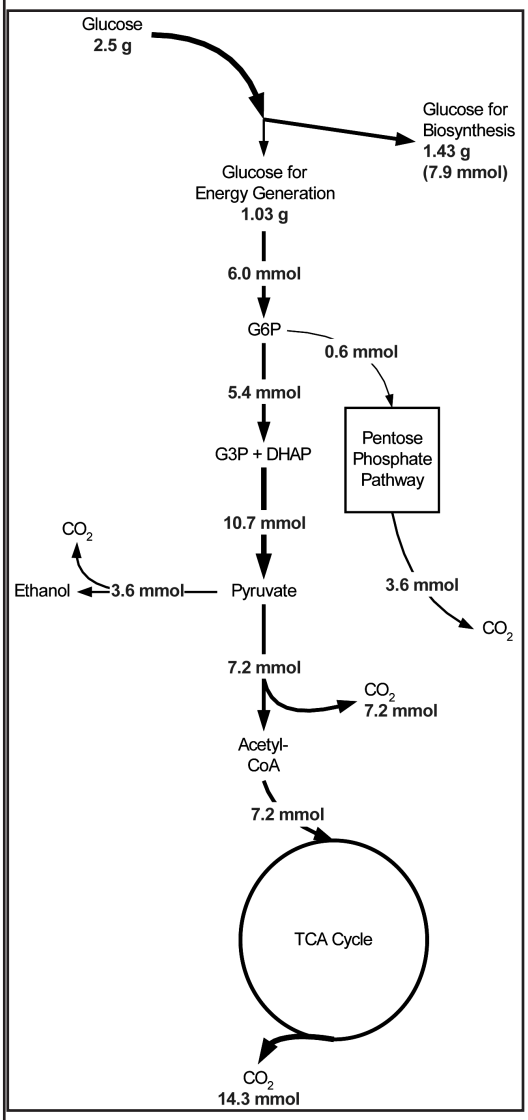


Figure 13. (left) *Fluxes among central metabolic pathways for biosynthesis, drawn based on Metstoich results.*

Figure 14. (below) *Fluxes among central metabolic pathways for energy generation purpose, drawn based on Metstoich results.*



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