Understanding the Practical Consequences of Metabolic Interactions – A Software Package for Teaching and Research

Wong, K W [1] ; Barford, J P [2] and Porter, J F [3]

[1] kelvwong@ust.hk
[2] barford@ust.hk
[3] kejep@ust.hk
Department of Chemical Engineering,
The Hong Kong University of Science and Technology

ABSTRACT

METSTOICH, a metabolite balancing software package, was developed for use in teaching metabolic pathways and their interactions. Based on the metabolism of Baker's Yeast, the package has been used to examine the relationship between cell yield, cell composition, P/O ratio, and energy (ATP) utilization during cell growth. After the simulation was developed, a number of problem sets were developed which targeted particular cellular interactions. These had increasing levels of difficulty. The simulation was then trialed in the postgraduate course BIEN502 (Biochemistry for Bioengineering). Initial trials indicated that the package provides a useful supplement to traditional methods in teaching metabolism. Student evaluation of the course indicated that the simulation was considered a very useful supplement to traditional teaching methods, and that it was easy to use and to understand. The simulation was supported by a large help file which included background theory, nomenclature and the problem sets. Some minor operational faults and some suggestions by the students for further improvement were incorporated into a revised simulation. This will be trialed further in CENG565 (Environmental Biotechnology) and CENG361 (Biochemical Engineering). In addition, a supplementary grant will allow it to be trialed in Biochemistry, where the more basic biochemical details will be focused upon.

Keywords

Education, simulation, metabolism, energetics, biochemical pathways

INTRODUCTION

This paper presents a summary of the initial development of METSTOICH, one such metabolite balancing software package. The program was developed as a teaching tool for undergraduate and graduate courses in chemical engineering, biochemistry and bioengineering at HKUST.

DEVELOPMENT OF METABOLITE BALANCING SOFTWARE PACKAGE

Unlike the more generic metabolism simulators, METSTOICH was initially developed for teaching purposes based on the metabolism of a specific yeast (Oura, 1972) under aerobic conditions. It focused on addressing the issue of energetics and efficiency of ATP usage. The results generated by METSTOICH are organized into different levels of metabolic detail, with illustrative reaction pathways included to make it more understandable to students. The underlying calculation package of METSTOICH was written in Excel with a front-end designed in Visual Basic. It runs on Microsoft Windows 98, 2000 and XP with Microsoft Office 2000 and XP. It can estimate the theoretical yield, or it can calculate the actual P/O ratio, YATP/X for experimental data with full detailed metabolism reports with illustrative reactions. These detailed reports can be exported to a Microsoft Excel workbook.

Based on the specified yeast metabolism, METSTOICH involves 168 anabolic reactions, 72 catabolic reactions and more than 170 chemical species and 16 branchpoint metabolites. A simplified metabolic pathway diagram of the major metabolites involved in both the catabolic and anabolic pathways is shown in Figure 1. Examples of typical ‘overall’ reactions for individual monomer/metabolites (amino acids) pathways are also shown in Barford (2003). Examples of detailed amino acids (monomer) compositions among proteins used in the above calculations are based on the compositions reported by Oura (1972) and are shown in Barford (2003). Enzyme concentrations, kinetic expressions, intermediate concentrations, and thermodynamics have not been incorporated in the current version.

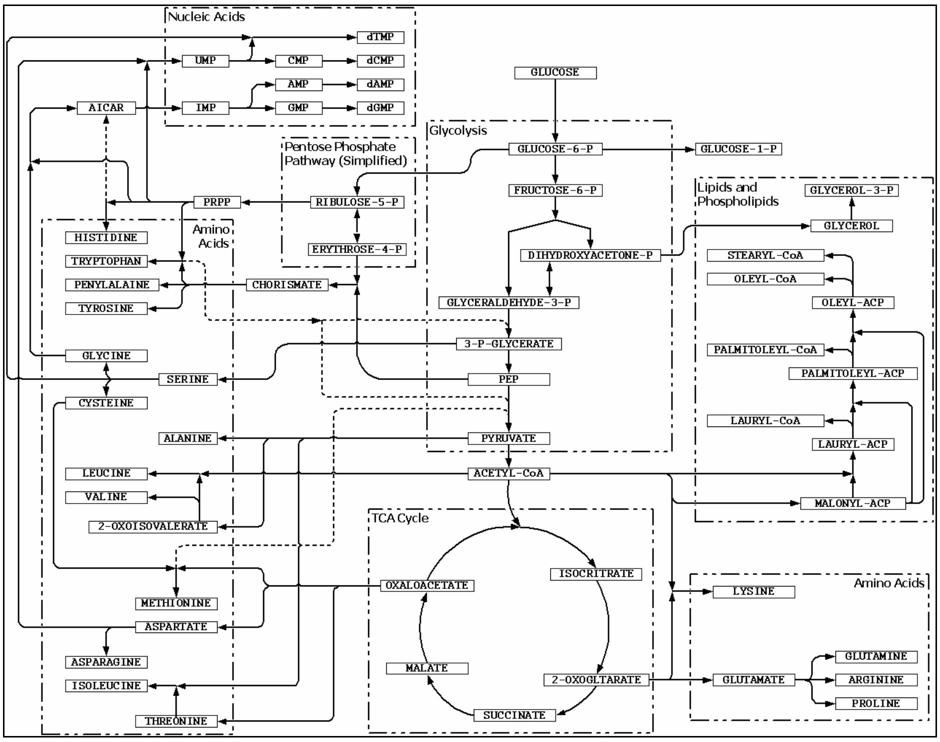

Figure 1. Central metabolic pathways and precursors for macromolecules (based on Oura, 1972)

A flowchart for the calculations in METSTOICH is shown in Figure 2. The amount of glucose required for a given biomass composition is back-calculated based on known reaction stoichiometry. The overall ATP requirement is estimated based on the individual ATP consumptions in anabolic processes with a specified ATP utilization efficiency. The ATP utilization efficiency is the fraction of the total ATP consumed that is used directly for cell growth (cell material manufacturing): $\eta = \frac{ATP_{Consumed,Anabolism}}{ATP_{Consumd,Total}}$. The difference between the total ATP consumed and the ATP used for cell growth, i.e., $ATP_{Consumed,Total} - ATP_{Consumed,Anabolism}$, is the ATP used for several cellular functions, such as to maintain concentration gradients of several chemical species across the cell membrane, active transport of materials, cell movement, futile cycle, enzyme turnover, DNA repair, etc. These activities are not well defined or quantified. All reducing power required/generated through any process is summed into a NAD(H) pool (overall NAD(P)H, NAD(H), FADH2 produced/consumed) and ATP generated/required by this NAD(H) pool is estimated using the P/O ratio. The overall ATP produced is the sum of ATP generated/required by the NAD(H) pool and ATP generated by the catabolic processes. The overall ATP produced is balanced against the estimated overall ATP requirement.

Cell yield can be estimated based on the amount of glucose required for both biomass composition and energy generation. YATP/X is defined in the literature as the amount of cell produced per mole of ATP generated in the energy-generation process. Consequently, it does not include the energy possibly produced in the manufacture of the intermediates before they are processed by the cell into monomers.

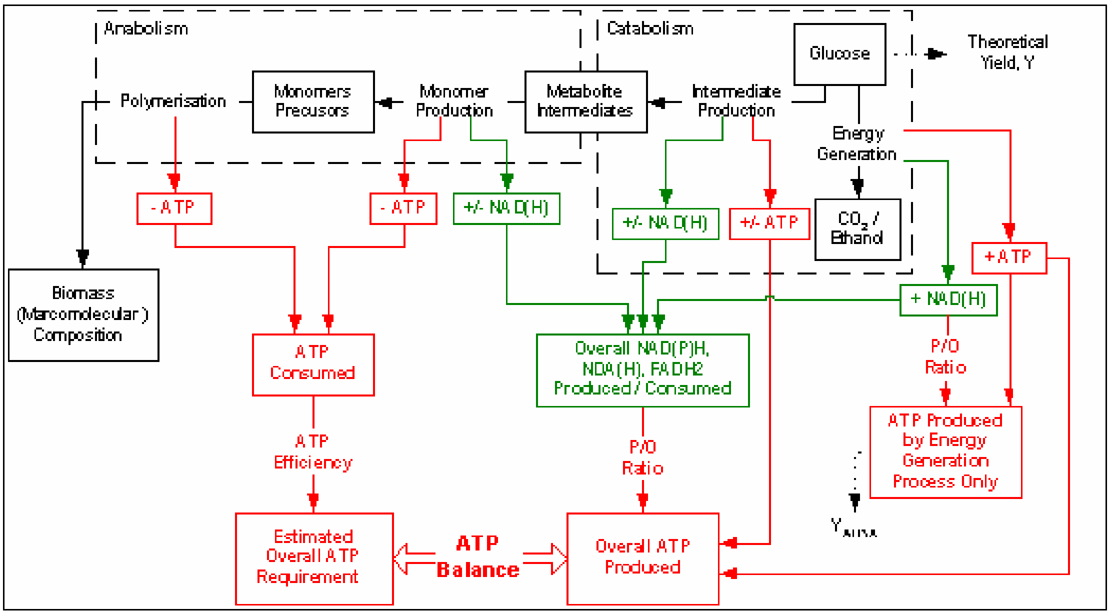

Figure 2. Strategy for metabolite balancing

RESULTS AND DISCUSSION

The values of major metabolic variables given by the simulation are consistent with typical values found in the literature (Table 1):

	Calculation Inputs and Results			Acceptable Range
P/O Ratio	2.00	2.00	2.00	2.00
ATP utilization efficiency	30%	20%	10%	N/A
g-glucose /100g-cell	159.80	184.22	221.33	222.22 – 181.82 *
Percent glucose used for:				
Biosynthesis	84.84%	72.59%	61.25%	54.0 –64.8% *
Energy generation	15.16%	26.41%	38.75%	46.0 – 35.2% *
Y (g cell / g glucose)	0.626	0.543	0.452	0.45 - 0.54 (Barford, 1991a, 1991b)
$Y_{ATP/X}$ (g cell / mol ATP)	25.43	18.01	7.19	6.29 – 9.86 *

Note: * - Estimated data, assuming biomass is composed of 48 wt% carbon.

Table 1. Comparison between model results and typical literature values

The simulation is linked to an interface calculator, which allows students to input the required data for a particular category of calculation type. This process is illustrated in Figures 3 and 4. Figure 3 shows the input interface and the calculation type illustrated is for a single set of data (Input Field 1 – 'Define mode of calculation') and a problem type related to Theoretical Cell Yield calculation (Input Field 2 – 'Specify type of result'). The input data for Figure 3 is blank. After data entry (Figure 4), the 'Get Results' button is used to execute the calculation (Input Field 4 – 'Execute calculation').

The results are then displayed in another interface and the user can then choose from three options for the type and extent of the data displayed. The first two options are illustrated in Figures 5 and 6. These correspond to the categories 'Cell Yield and Energetics' and 'Fate of Glucose'. The first category provides information on cell from substrate yield, cell from ATP yield and the production and use of ATP and NAD(H) for catabolism and anabolism. It also estimates the amount of ATP required for Cellular Functions other than cell growth. The second category provides information on the fate of glucose and, in particular, whether it is used for energy generation or cell manufacture and the extent to which the various energy-generating pathways (Glycolysis, Pentose Phosphate Pathway, TCA Cycle and Fermentation) are used. The third output category available is 'Full Output'. This gives the details of every single output simulation. This is not illustrated in this paper. This option is for the advanced user and is very extensive.

Four problem sets were developed for the trial of this simulation – Theoretical Cell Yield Calculation, Experimental Yield Calculation, Theoretical PO Ratio Calculation and Experimental PO Ration Calculation. The first of these options is shown in Figure 7.

The simulation has an extensive help file which contains Problem Sets (Figure 7), Background Information (Figure 8), Nomenclature (Figure 9), Representative web pages and Reference Materials (Figure 10) and a User Manual (Figure 11).

The simulation was then trialed in the postgraduate course BIEN502 (Biochemistry for Bioengineering). Initial trials indicated that the package provides a useful supplement to traditional methods in teaching metabolism. Student evaluation of the course indicated that the simulation was considered a very useful supplement to traditional teaching methods, and that it was easy to use and to understand. The simulation was supported by a large help file which included background theory, nomenclature and the problem sets. Some minor operational faults and some suggestions by the students for further improvement were incorporated into a revised simulation. This will be trialed further in CENG565 (Environmental Biotechnology) and CENG361 (Biochemical Engineering). In addition, a supplementary grant will allow it to be trialed in Biochemistry, where the more basic biochemical details will be focused upon.

CONCLUSIONS

Initial results from METSTOICH, a metabolite-balancing package developed at HKUST, indicate that such a package is very useful as a teaching tool to allow students to understand metabolic pathways and their interaction. Specific effects, for example the variation in cell growth energetics with variations in the cell compositions, may be studied. Student evaluations have been very positive.

ACKNOWLEDGEMENTS

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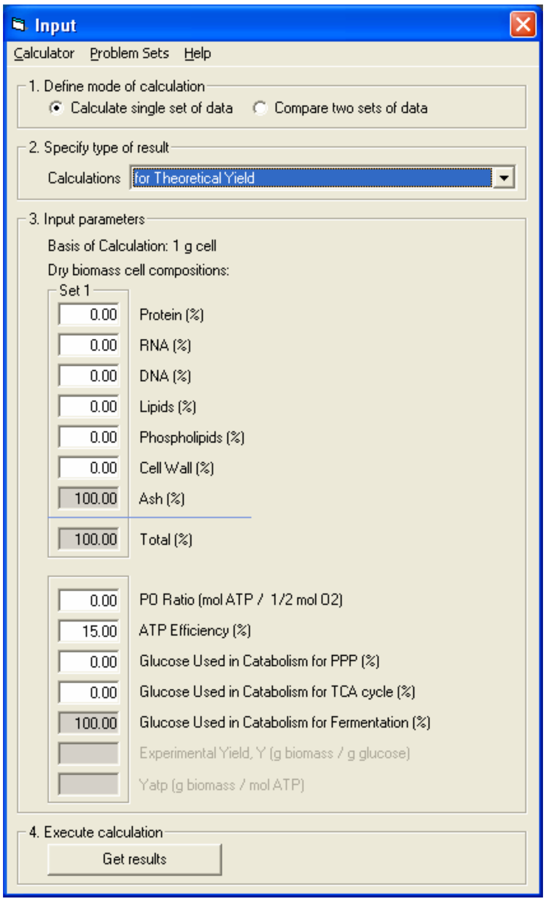


Figure 3. Input interface (Blank)

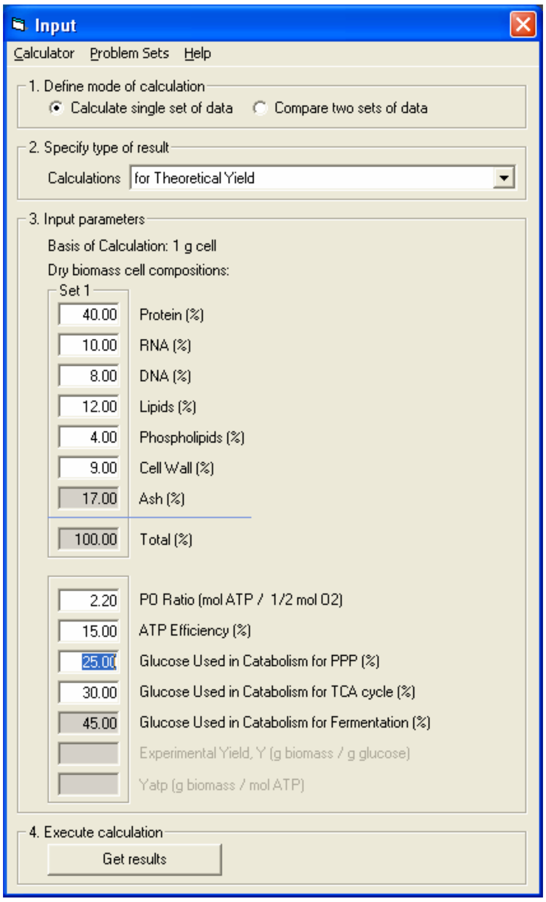


Figure 4. Input interface – data entry for problem Set 1

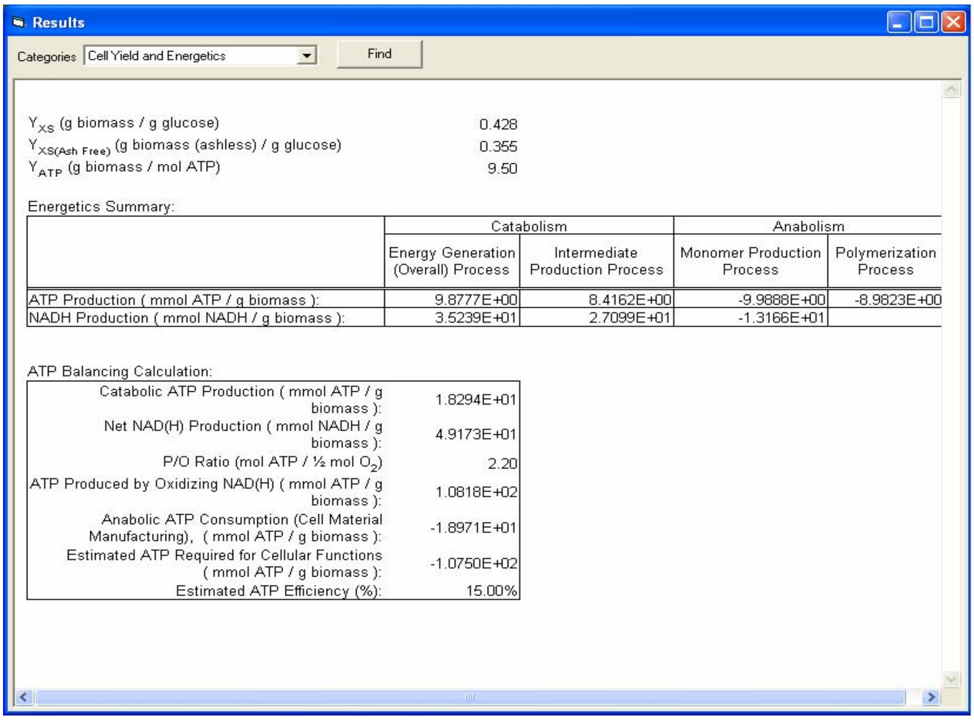
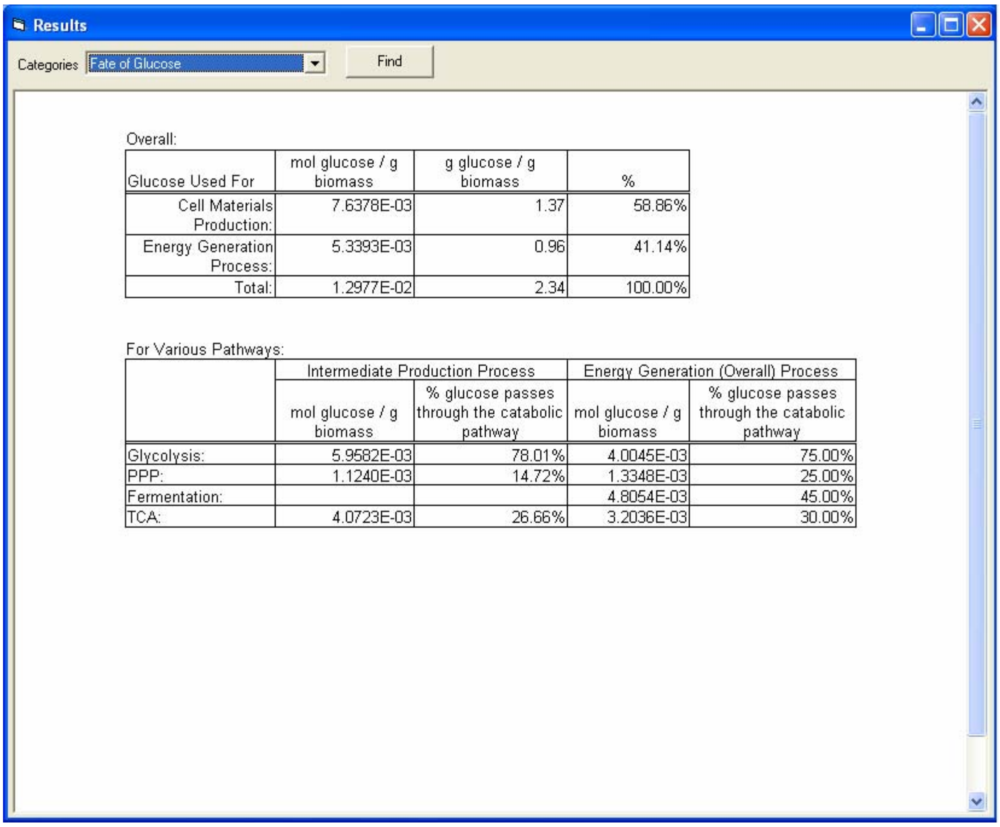


Figure 5. Calculation result – 'Cell Yield and Energetics'



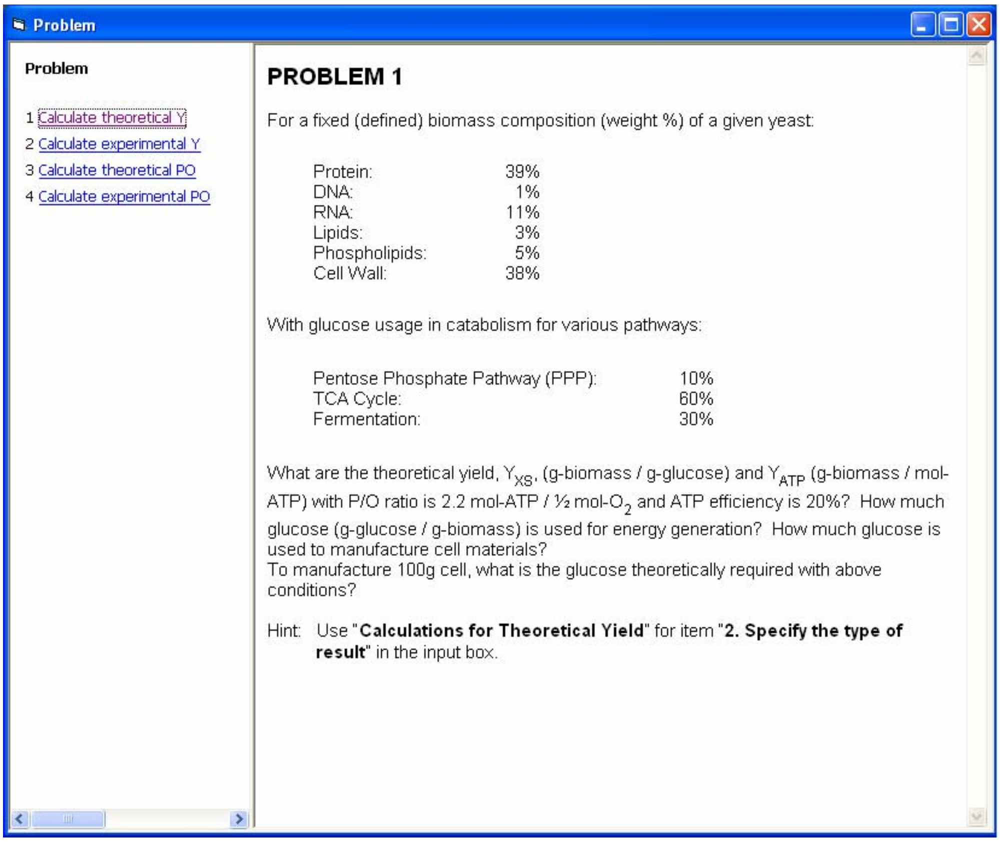
Overall:

Glucose Used For	mol glucose / g biomass	g glucose / g biomass	%
Cell Materials Production:	7.6378E-03	1.37	58.86%
Energy Generation Process:	5.3393E-03	0.96	41.14%
Total:	1.2977E-02	2.34	100.00%

For Various Pathways:

	Intermediate Production Process		Energy Generation (Overall) Process	
	mol glucose / g biomass	% glucose passes through the catabolic pathway	mol glucose / g biomass	% glucose passes through the catabolic pathway
Glycolysis:	5.9582E-03	78.01%	4.0045E-03	75.00%
PPP:	1.1240E-03	14.72%	1.3348E-03	25.00%
Fermentation:			4.8054E-03	45.00%
TCA:	4.0723E-03	26.66%	3.2036E-03	30.00%

Figure 6. Calculation result – 'Fate of Glucose'



PROBLEM 1

For a fixed (defined) biomass composition (weight %) of a given yeast:

Protein:	39%
DNA:	1%
RNA:	11%
Lipids:	3%
Phospholipids:	5%
Cell Wall:	38%

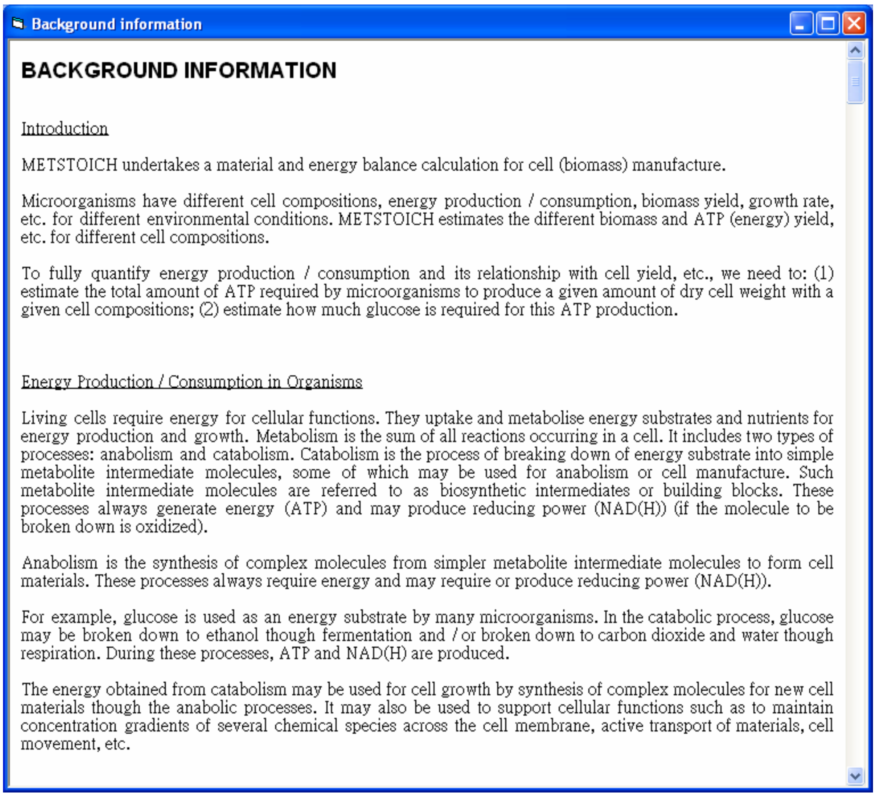
With glucose usage in catabolism for various pathways:

Pentose Phosphate Pathway (PPP):	10%
TCA Cycle:	60%
Fermentation:	30%

What are the theoretical yield, Y_{XS}, (g-biomass / g-glucose) and Y_{ATP} (g-biomass / mol-ATP) with P/O ratio is 2.2 mol-ATP / ½ mol-O_2 and ATP efficiency is 20%? How much glucose (g-glucose / g-biomass) is used for energy generation? How much glucose is used to manufacture cell materials?
To manufacture 100g cell, what is the glucose theoretically required with above conditions?

Hint: Use "**Calculations for Theoretical Yield**" for item "**2. Specify the type of result**" in the input box.

Figure 7. Problem sets for the trial of METSTOICH


Background information

BACKGROUND INFORMATION

Introduction

METSTOICH undertakes a material and energy balance calculation for cell (biomass) manufacture.

Microorganisms have different cell compositions, energy production / consumption, biomass yield, growth rate, etc. for different environmental conditions. METSTOICH estimates the different biomass and ATP (energy) yield, etc. for different cell compositions.

To fully quantify energy production / consumption and its relationship with cell yield, etc., we need to: (1) estimate the total amount of ATP required by microorganisms to produce a given amount of dry cell weight with a given cell compositions; (2) estimate how much glucose is required for this ATP production.

Energy Production / Consumption in Organisms

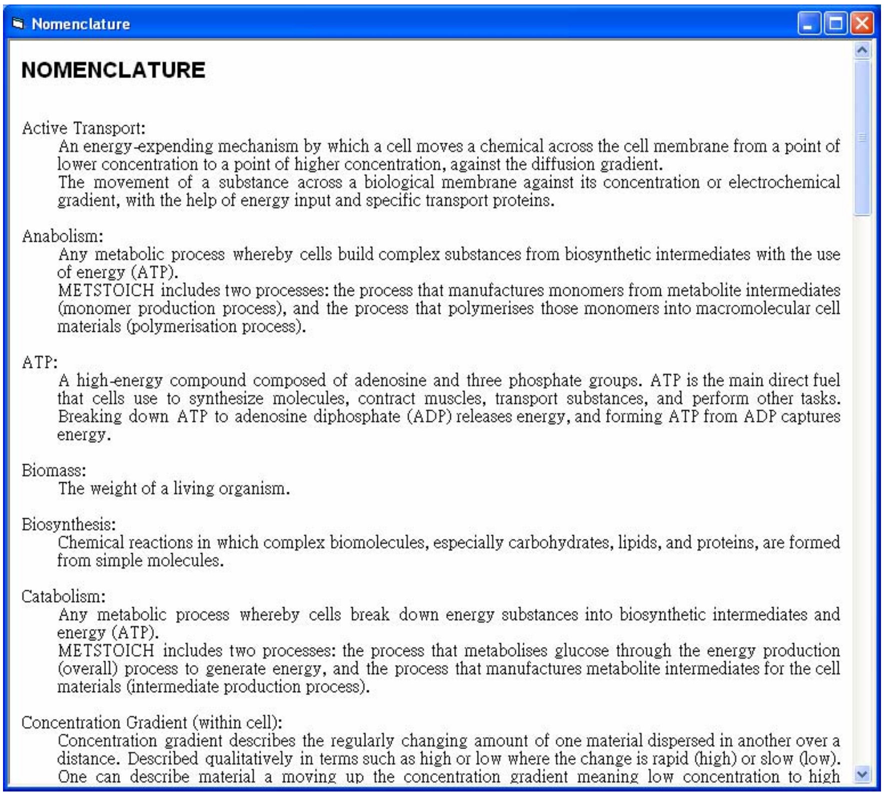
Living cells require energy for cellular functions. They uptake and metabolise energy substrates and nutrients for energy production and growth. Metabolism is the sum of all reactions occurring in a cell. It includes two types of processes: anabolism and catabolism. Catabolism is the process of breaking down of energy substrate into simple metabolite intermediate molecules, some of which may be used for anabolism or cell manufacture. Such metabolite intermediate molecules are referred to as biosynthetic intermediates or building blocks. These processes always generate energy (ATP) and may produce reducing power (NAD(H)) (if the molecule to be broken down is oxidized).

Anabolism is the synthesis of complex molecules from simpler metabolite intermediate molecules to form cell materials. These processes always require energy and may require or produce reducing power (NAD(H)).

For example, glucose is used as an energy substrate by many microorganisms. In the catabolic process, glucose may be broken down to ethanol though fermentation and / or broken down to carbon dioxide and water though respiration. During these processes, ATP and NAD(H) are produced.

The energy obtained from catabolism may be used for cell growth by synthesis of complex molecules for new cell materials though the anabolic processes. It may also be used to support cellular functions such as to maintain concentration gradients of several chemical species across the cell membrane, active transport of materials, cell movement, etc.

Figure 8. Background information file to help students understand the concepts behind METSTOICH


Nomenclature

NOMENCLATURE

Active Transport:
An energy-expending mechanism by which a cell moves a chemical across the cell membrane from a point of lower concentration to a point of higher concentration, against the diffusion gradient.
The movement of a substance across a biological membrane against its concentration or electrochemical gradient, with the help of energy input and specific transport proteins.

Anabolism:
Any metabolic process whereby cells build complex substances from biosynthetic intermediates with the use of energy (ATP).
METSTOICH includes two processes: the process that manufactures monomers from metabolite intermediates (monomer production process), and the process that polymerises those monomers into macromolecular cell materials (polymerisation process).

ATP:
A high-energy compound composed of adenosine and three phosphate groups. ATP is the main direct fuel that cells use to synthesize molecules, contract muscles, transport substances, and perform other tasks. Breaking down ATP to adenosine diphosphate (ADP) releases energy, and forming ATP from ADP captures energy.

Biomass:
The weight of a living organism.

Biosynthesis:
Chemical reactions in which complex biomolecules, especially carbohydrates, lipids, and proteins, are formed from simple molecules.

Catabolism:
Any metabolic process whereby cells break down energy substances into biosynthetic intermediates and energy (ATP).
METSTOICH includes two processes: the process that metabolises glucose through the energy production (overall) process to generate energy, and the process that manufactures metabolite intermediates for the cell materials (intermediate production process).

Concentration Gradient (within cell):
Concentration gradient describes the regularly changing amount of one material dispersed in another over a distance. Described qualitatively in terms such as high or low where the change is rapid (high) or slow (low).
One can describe material a moving up the concentration gradient meaning low concentration to high

Figure 9. Nomenclature file to explain terminologies relevant to metabolite balancing

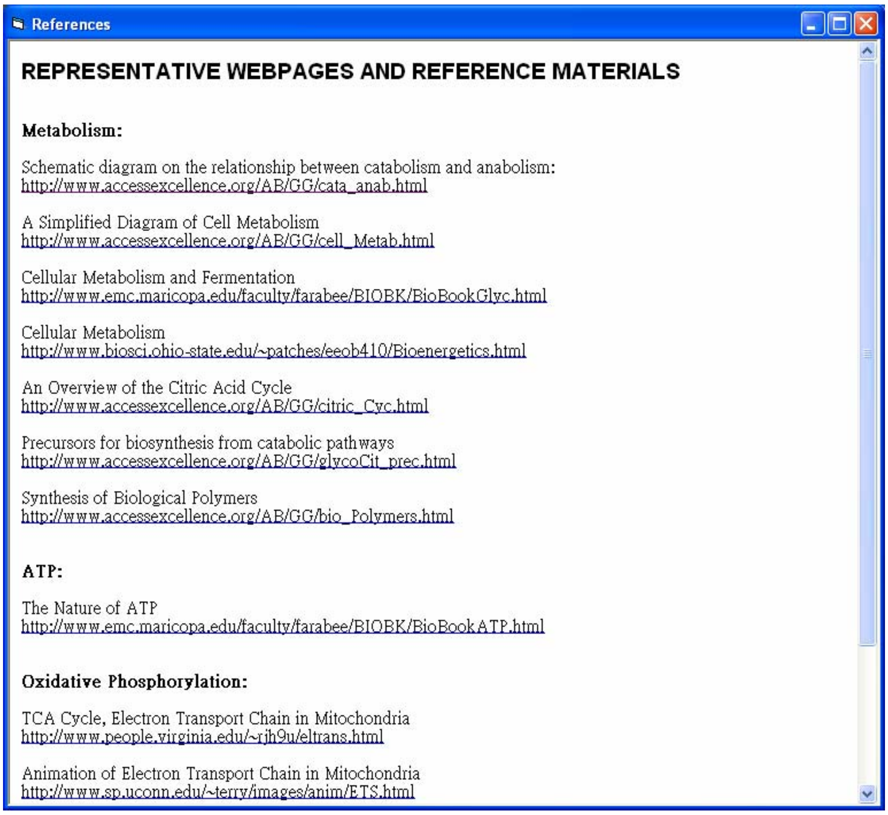


Figure 10. Representative web pages and reference material to allow students to further study related topics

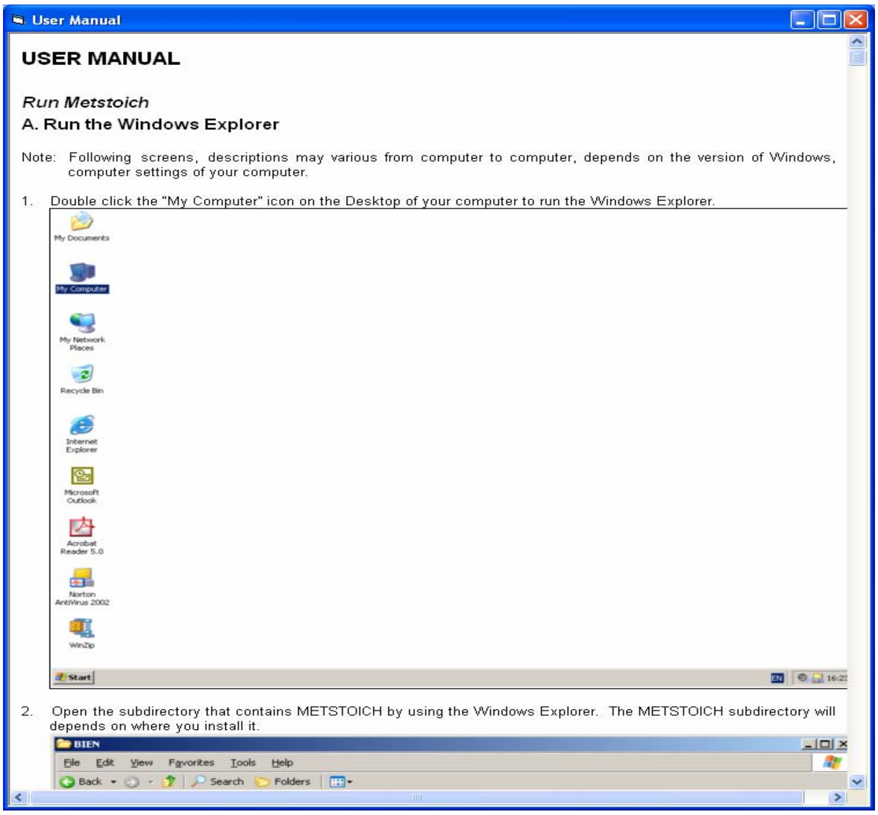


Figure 11. METSTOICH user manual