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

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Software Enabling the Study of the Practical Consequences of Metabolic Pathways Interactions – METSTOICH

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) utilisation during cell growth.

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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. Detailed output reports can be exported to a Microsoft Excel workbook.

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Based on the specified yeast metabolism, METSTOICH involves 168 anabolic reactions, 72 catabolic reactions and more than 170 chemical species and 16 branch point metabolites.

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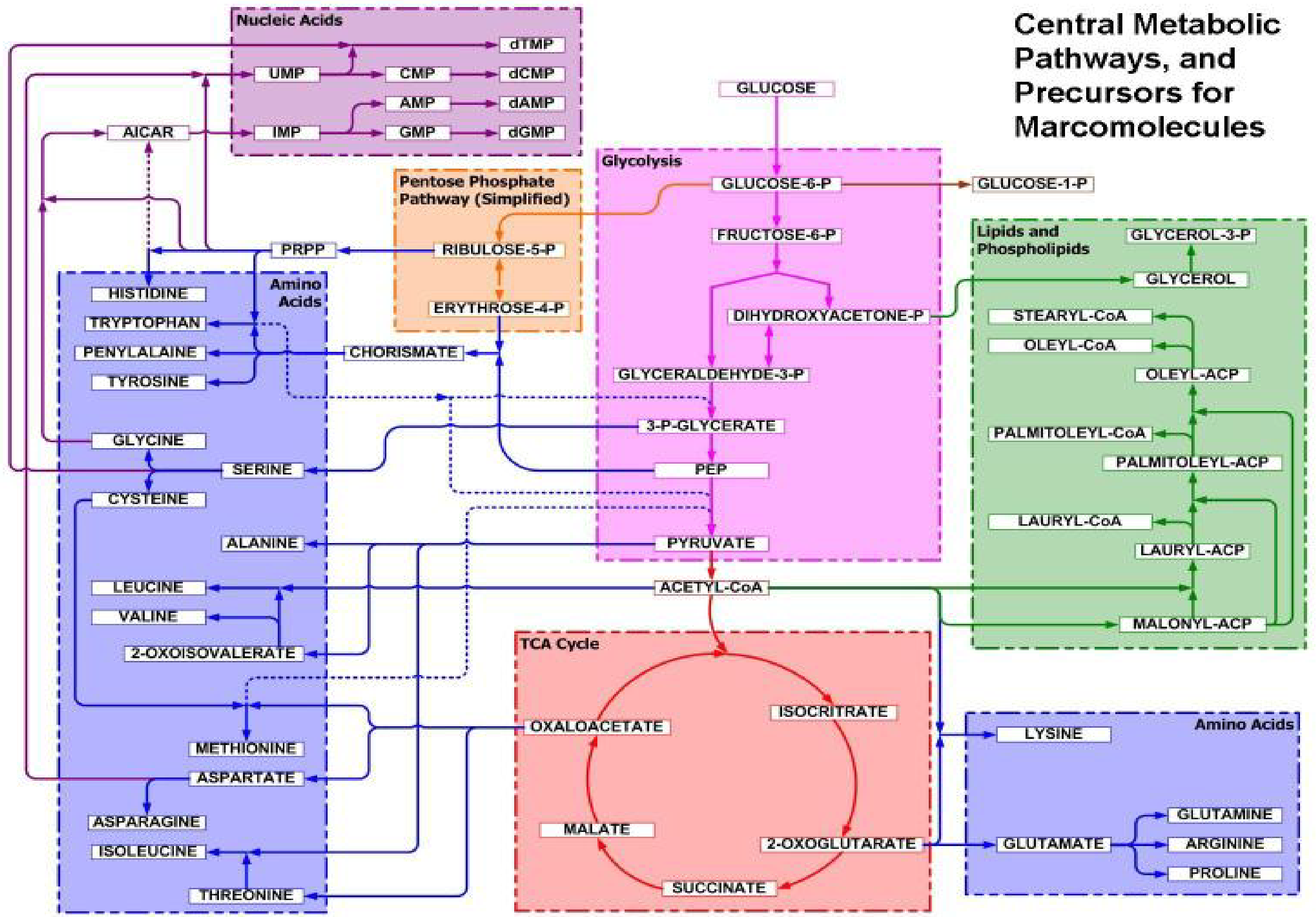

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Strategy for Metabolite Balancing

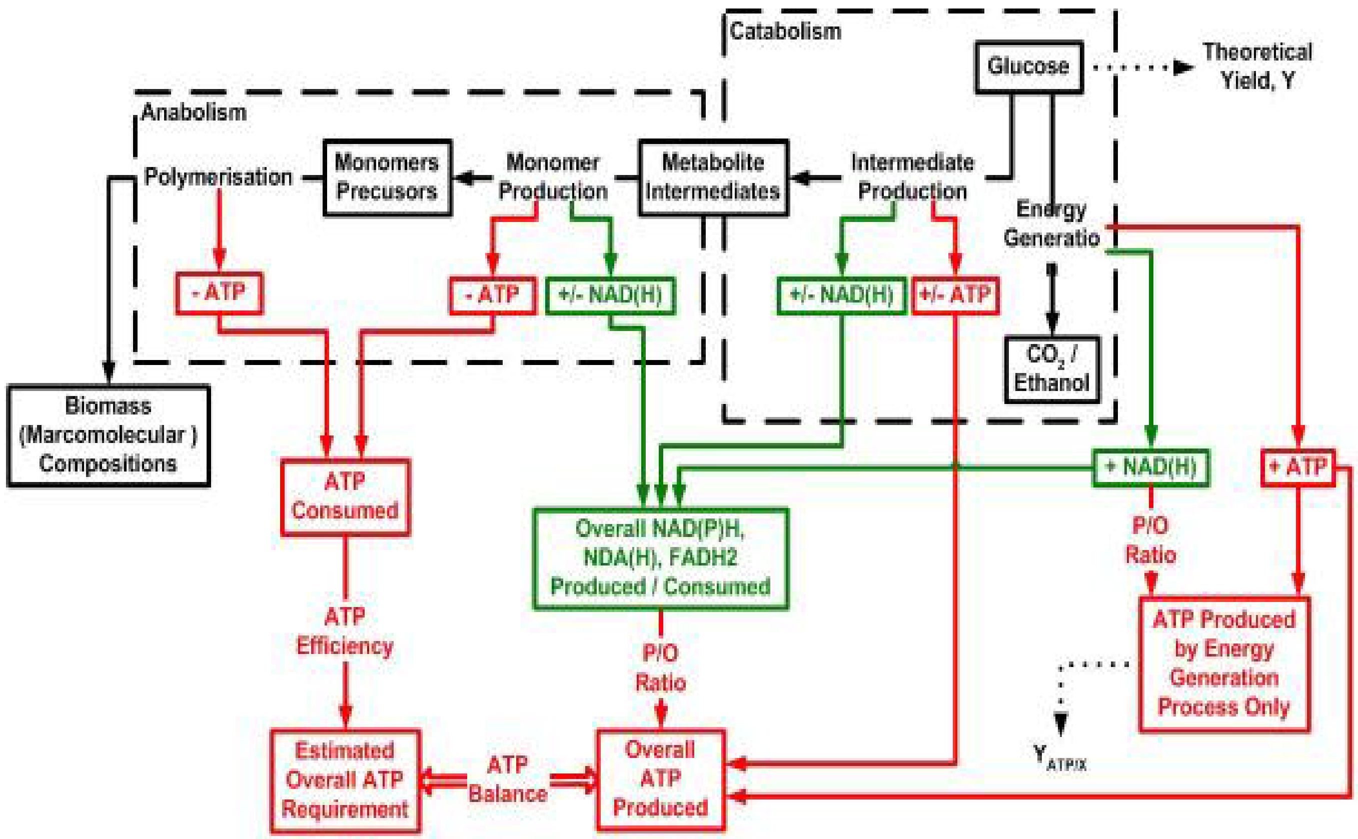

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Table 1. Comparison Between Model Results and Typical Literature Values

	Calculation Inputs and Results			Acceptable Range
P/O Ratio	2.00	2.00	2.00	2.00
ATP utilisation 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.

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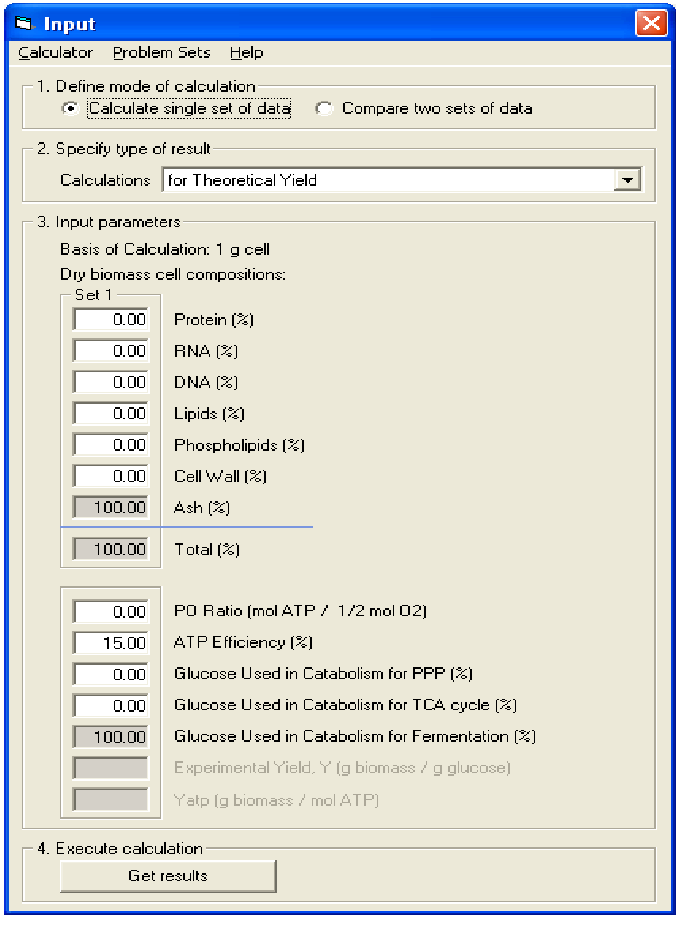

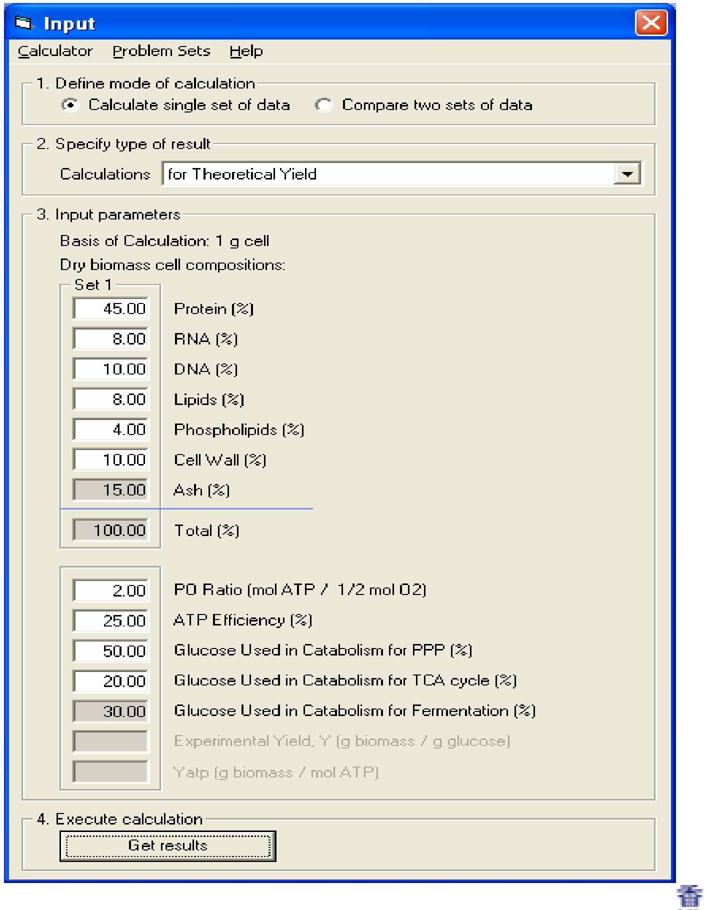

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Results

Categories: Cell Yield and Energetics | Find

Y_{XS} (g biomass / g glucose) 0.568

$Y_{XS(Ash\ Free)}$ (g biomass (ashless) / g glucose) 0.483

Y_{ATP} (g biomass / mol ATP) 21.35

Energetics Summary:

	Catabolism		Anabolism	
	Energy Generation (Overall) Process	Intermediate Production Process	Monomer Production Process	Polymerization Process
ATP Production (mmol ATP / g biomass):	2.0247E+00	7.7705E+00	-9.2413E+00	-9.9575E+00
NADH Production (mmol NADH / g biomass):	1.8897E+01	2.6492E+01	-1.1890E+01	

ATP Balancing Calculation:

Catabolic ATP Production (mmol ATP / g biomass):	9.7952E+00
Net NAD(H) Production (mmol NADH / g biomass):	3.3500E+01
P/O Ratio (mol ATP / ½ mol O_2)	2.00
ATP Produced by Oxidizing NAD(H) (mmol ATP / g biomass):	6.7000E+01
Anabolic ATP Consumption (Cell Material Manufacturing), (mmol ATP / g biomass):	-1.9199E+01
Estimated ATP Required for Cellular Functions (mmol ATP / g biomass):	-5.7596E+01
Estimated ATP Efficiency (%):	25.00%

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Results

Categories: Fate of Glucose   Find

Overall:

Glucose Used For	mol glucose / g biomass	g glucose / g biomass	%
Cell Materials Production:	7.5315E-03	1.36	77.00%
Energy Generation Process:	2.2497E-03	0.40	23.00%
Total:	9.7812E-03	1.76	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:	5.7196E-03	75.94%	1.1248E-03	50.00%
PPP:	1.1946E-03	15.86%	1.1248E-03	50.00%
Fermentation:			1.3498E-03	30.00%
TCA:	4.5065E-03	29.92%	8.9987E-04	20.00%

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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).

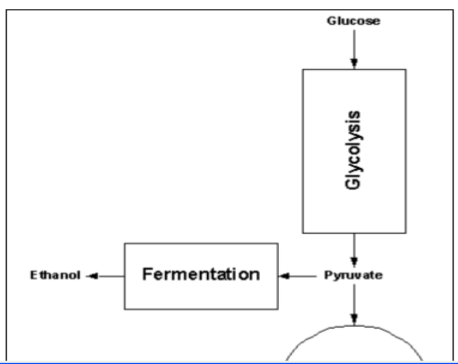
Anabolism is the synthesis of complex molecules from simpler metabolite intermediate molecules to form cell materials. These processes always require energy and may require or produce reducing power (NAD(H)).

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.

Energy Generation Process in Catabolism

Catabolism may occur by a number of different pathways, the two predominant ones being glycolysis followed by either fermentation and / or respiration (Figure 1).



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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 concentration or down the concentration gradient meaning from high concentration to low concentration.

Concentration gradients exists in cell, e.g. at cell membrane, among different cell compartments, etc.

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REPRESENTATIVE WEBPAGES AND REFERENCE MATERIALS

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Schematic diagram on the relationship between catabolism and anabolism:
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A Simplified Diagram of Cell Metabolism
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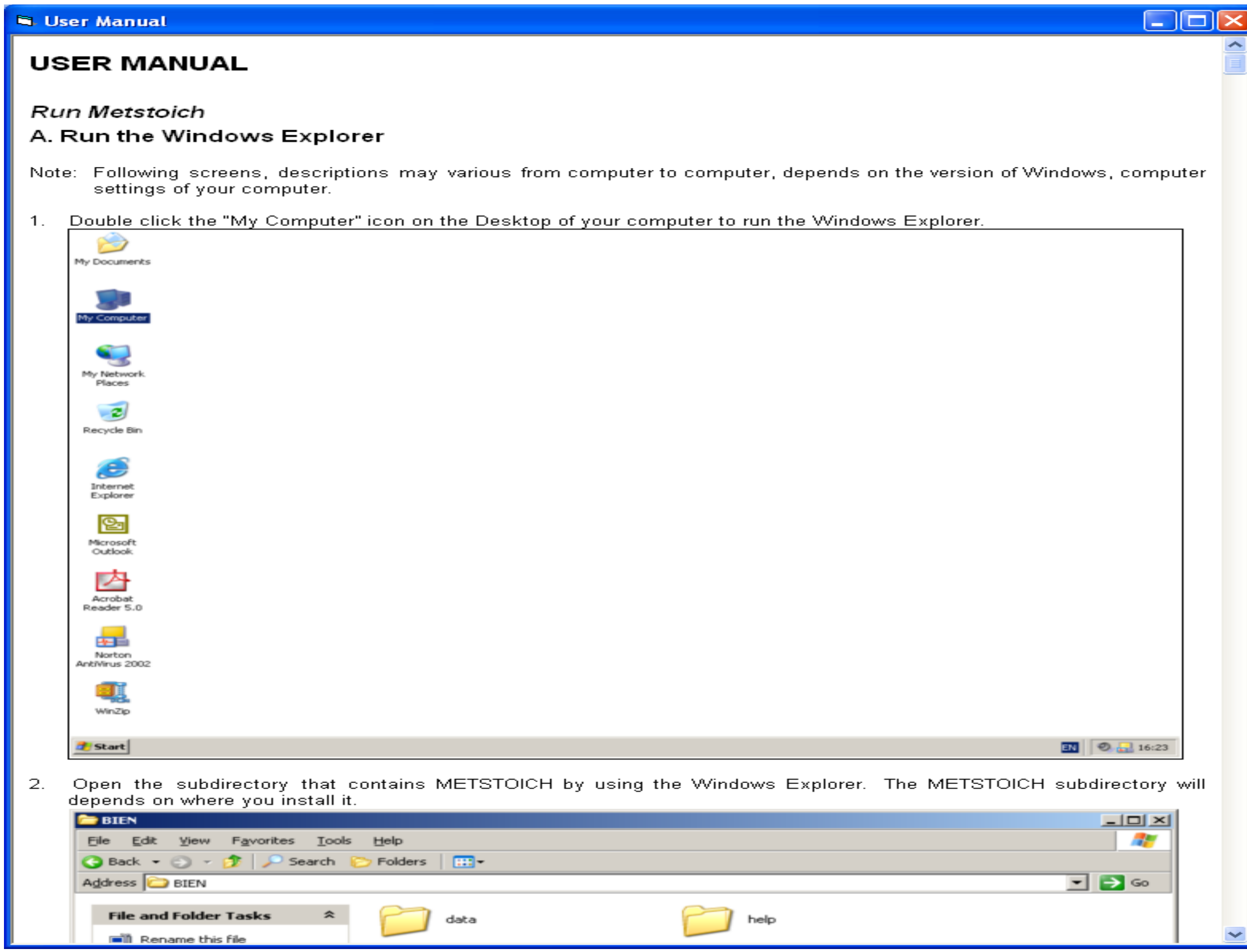

USER MANUAL

Run Metstoich

A. Run the Windows Explorer

Note: Following screens, descriptions may various from computer to computer, depends on the version of Windows, computer settings of your computer.

1. Double click the "My Computer" icon on the Desktop of your computer to run the Windows Explorer.

2. Open the subdirectory that contains METSTOICH by using the Windows Explorer. The METSTOICH subdirectory will depends on where you install it.

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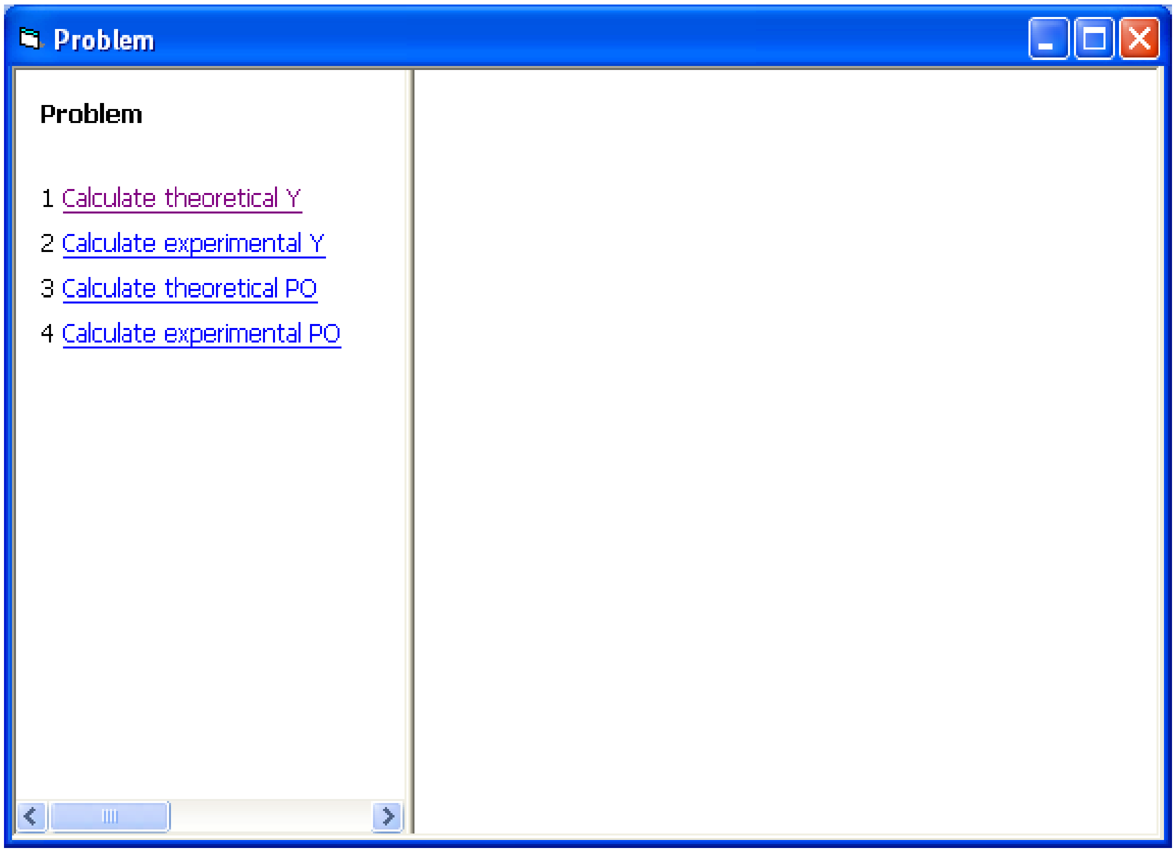

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Results

Categories: All reaction details

Overall | Protein | DNA RNA | Lipids | Cell Wall | Biosynthesis 1 | Biosynthesis 2 | Energy Generation | Energy Balance

0 Dry Biomass Compositions

	Weight %
Protein	45.00
RNA	8.00
DNA	10.00
Lipids	8.00
Phospholipids	4.00
Cell Wall	10.00
Ash	15.00
Total	100
Total (Ashless):	85

1 Other Parameters

Assume weight of biomass is given (g biomass)	1.00
PO Ratio:	2.00
Anabolic ATP Efficiency:	25.00%
Precent of Glucose used in Energy Generation (%):	
For PPP:	50.0%
For TCA:	20.0%
For Fermentation:	30.0%
Problem Type:	Calculate theoretical yield

2 Marcomolecules

Protein	Weight (g acid / g biomass)	Weight Ratio of Amino Acids among Protein	Molecular Weight	Mole (mol acid / g biomass)	Mole Ratio of Amino Acids among Protein
Amino Acids	0.4500	1.1681	136.75	4.1949E-03	1.0000
Alanine (Ala)	0.0394	0.1022	89	5.5453E-04	0.1322
Arginine (Arg)	0.0270	0.0700	174	1.7286E-04	0.0412
Asparagine (Asn)	0.0129	0.0336	132	1.1354E-04	0.0271
Aspartate (Asp)	0.0381	0.0990	133	3.3164E-04	0.0791
Cysteine (Cys)	0.0008	0.0020	121	7.4804E-06	0.0018
Glutamate (Glu)	0.0428	0.1110	147	3.3149E-04	0.0790
Glutamine (Gln)	0.0148	0.0385	146	1.1587E-04	0.0276
Glycine (Gly)	0.0210	0.0545	75	3.6834E-04	0.0878
Histidine (His)	0.0099	0.0257	155	7.2268E-05	0.0172
Isoleucine (Ile)	0.0243	0.0632	131	2.1546E-04	0.0514
Leucine (Leu)	0.0374	0.0972	131	3.3138E-04	0.0790
Lysine (Lys)	0.0403	0.1046	146	3.1481E-04	0.0750
Methionine (Met)	0.0073	0.0189	149	5.5581E-05	0.0132
Phenylalamine (Phe)	0.0213	0.0553	165	1.4492E-04	0.0345
Proline (Pro)	0.0183	0.0474	115	1.8825E-04	0.0449

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0

	Mole (mol precursor / g biomass)	Mole Ratio of Precursors among Lipids / Phospholipids
Neutral Lipids	4.2538E-04	1.000
Glycerol	1.0632E-04	0.250
Lauric Acid	9.5630E-05	0.225
Palmitoleic Acid	1.5969E-04	0.375
Oleic Acid	6.3747E-05	0.150
Phospholipids	1.5015E-04	1.000
Glycerol-3-Phosphate	5.0000E-05	0.333
Stearic Acid	1.0015E-04	0.667

1

Stearic Acid (mol / g biomass):	Demand	Supply	Total (Demand)
For Phospholipids	1.0015E-04		1.0015E-04

Oleyl-S-ACP	NADPH2	→	Stearyl-S-ACP	NADP
1.0015E-04	1.0015E-04		1.0015E-04	1.0015E-04
Stearyl-S-ACP	CoA-SH	→	Stearyl-S-CoA	ACP-SH
1.0015E-04	X00		1.0015E-04	X00

Stearic Acid Material Summary (mol / g biomass)	Produced	Consumed	Net (Produced)
Oleyl-S-ACP		1.0015E-04	-1.0015E-04
Stearyl-S-CoA	1.0015E-04		1.0015E-04

Stearic Acid Energetics Summary (mol / g biomass)	Produced	Consumed	Net (Produced)
NADPH2		1.0015E-04	-1.0015E-04
NADP	1.0015E-04		1.0015E-04

2

Glycerol-3-Phosphate (mol / g biomass):	Demand	Supply	Total (Demand)
For Phospholipids	5.0000E-05		5.0000E-05

Glycerol	ATP	→	Glycerol-3-Phosphate	ADP
5.0000E-05	5.0000E-05		5.0000E-05	5.0000E-05

Glycerol-3-Phosphate Material Summary (mol / g biomass)	Produced	Consumed	Net (Produced)
Glycerol		5.0000E-05	-5.0000E-05
Glycerol-3-Phosphate	5.0000E-05		5.0000E-05

Glycerol-3-Phosphate Energetics Summary (mol / g biomass)	Produced	Consumed	Net (Produced)
ATP		5.0000E-05	-5.0000E-05
ADP	5.0000E-05		5.0000E-05

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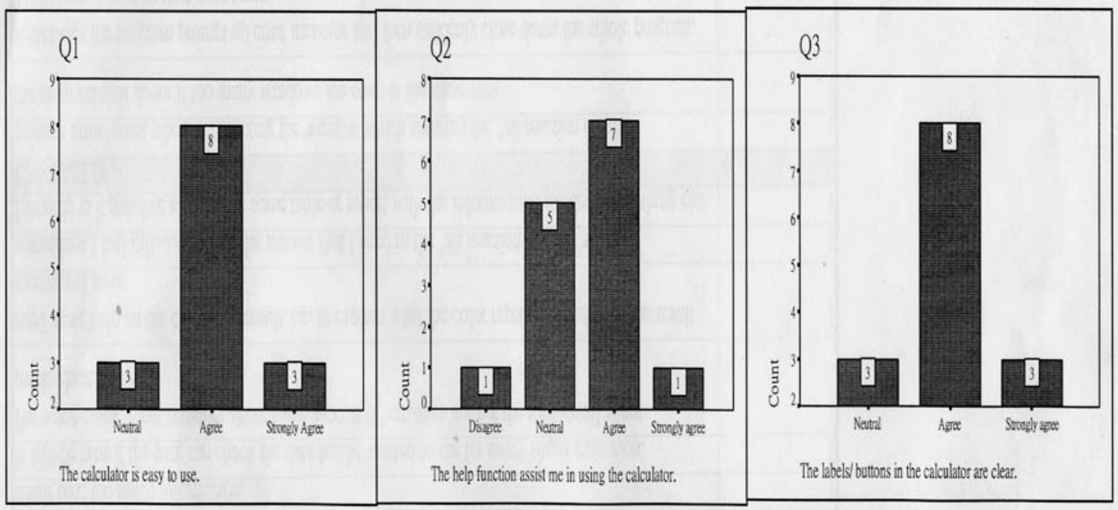
	Count	Mean	Standard Deviation
Q1. The calculator is easy to use.	14	4.0	0.7
Q2. The help function assist me in using the calculator.	14	3.6	0.8
Q3. The labels/ buttons in the calculator are clear.	14	4.0	0.7



The calculator is easy to use.
The help function assist me in using the calculator.
The labels/ buttons in the calculator are clear.

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Q4 & Q5: Difficulties in using the calculator & suggested area for enhancement

Comments	Count
PROBLEM WITH DISPLAY OF RESULTS	6
result box: too much information.	1
It will be nice if the user can check the data he/she wanted to see for every single execution.	1
For some cases, I just consider about some aspects or variables but not the total detail about metabolism pathway	1
need more help on the calculated result. i.e. to explain what the color represents and what the result means and so on	1
sometimes I feel difficult to find the answer that I need in the "all reaction details" result	1
I wonder is it possible to generate some table or report with the information I needed but nothing else.	1
UNSTABLE	4
runtime errors exist when maximizing the window which showing the "all reaction details"	1
the program shut down if too many windows are open at the same time	1
sometimes the program prompt up error message and then suddenly close down the whole program. That means I have to start over again.	1
maximizing "result window" can shut the program off	1
CANNOT SWITCH / END TASK WHEN RUNNING RESULT	4
I cannot switch to 'help' or other section of the program during the processing, even a simple click. Otherwise, the program will end abnormally	1
while processing if I press any button of the calculator, usually it will die. It's not rubust enough.	1
it should enable the users to close down the program when it is running	1
I hope I can stop processing whenever I find I made a mistake	1
SLOW COMPUTATION SPEED	3
the computation speed is somewhat too slow in some case	1
running the program for the last quesiton (Q4) took more than 7 mins	1

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it's too slow when calculating		1
NOT WORKING UNDER WINXP	2	
it seems cannot run under winxp		1
always has a sudden termination of the program in XP OS		1
POSITIVE COMMENT	1	
It is good if user can compare more than 2 sets of data in a time		1
OTHERS	7	
increase precision from 1.00 to 1.000		1
there's no index in help files, very inconvenient		1
and I hope when we fill in the input parameters. "Enter" can help us from one blank to another, just like excel, so I can finish it all by keyboard, and needn't transfer my right hand from keyboard to mouse and from mouse to keyboard		1
an icon for the program should be existed in the task bar. We can only see the icon in the task bar after clicking "calculate result" button		1
the check on whether "Dry biomass cell compositions" total exceeds 100% or not should be done when users push the button "Get result". Otherwise, it is not convenient to modify the composition.		1
the default scrolling of result window imposes great inconvenience		1
difficulty arises mostly from unfamilarity with fementation terminology		1
Total	27	

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Conclusions:

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. 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.

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