METSTOICH Teaching Quantitative Metabolism and Energetics in Biochemical Engineering

KELVIN W.W. WONG AND JOHN P. BARFORD

iochemistry 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

The Hong Kong University of Science and Technology • Clear Water Bay, Kowloon, HONG KONG

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.

John Barford is an associate professor of chemical engineering in the Department of Chemical and Biomolecular engineering at HKUST. He received his B.E. and Ph.D. from U.N.S.W. (Sydney). He teaches biomolecular engineering, environmental biotechnology, and biochemical engineering. His research interests include environmental biotechnology,

bio micro systems, and metabolic engineering and simulation of biological systems.

Kelvin Wong is a project assistant in the Department of Chemical and Biomolecular Engineering at HKUST. He received his B.Eng. and M.Phil. from HKUST and M.Sc. from the University of Sheffield. He develops Excel VBA programming coursework for the chemical engineering curriculum.

© Copyright ChE Division of ASEE 2010

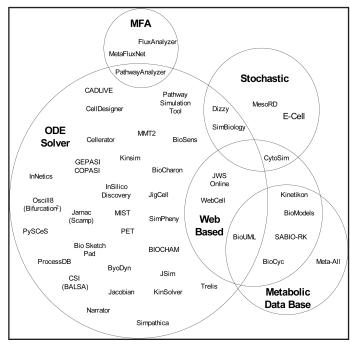


Parameters a	as Input and Ou	TABLE 1 tput for Various (Calculation Mo	des in Metstoich
		Pro	blem Types	
Parameters	(a) Theoretical Y _{xs}	(b) Experimental Y _{xs}	(c) Predefined Y _{X/ATP}	(d) Experimental Y_{xs} with Predefined $Y_{x/ATP}$
(1) Cell composi- tions	Input	Input	Input	Input
(2) Glucose usage for energy gen- eration 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.



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 pro-

vide 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.

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

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;*
- 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_{yy} ; 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, $Y_{y(ATP)}$.

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

Vol. 44, No. 2, Spring 2010

≒. Input Calculator Problem Sets H	elp	_	×
☐ 1. Define mode of calculation	n		
Calculate Single Set	of Data		
C Compare Two Sets of	of Data for Changes in Marco	molecular Compositions	
C Compare Two Sets o	of Data for Changes in Detaile	d Compositions	
2. Specify type of result			
	retical Yield		7
	retical Yield		
3. Input parameter based or	n Experimental Yield n Fixed Yatp		
Basis of Calculation y c	n both Experimental Yield and	f Fixed Yatp	
Cell composition Carbon	source Electron acceptor	Energy generation & other parameters	1
- Dry biomass cell comp	ositions:		
Set 1			
0.00	Protein (%)	Detailed Compositions	
0.00	RNA (%)	Detailed Compositions	
0.00	DNA (%)	Detailed Compositions	
0.00	Lipids (%)	Detailed Compositions	
0.00	Phospholipids (%)	Detailed Compositions	
0.00	Cell Wall (%)	Detailed Compositions	
100.00	Ash (%)		
100.00	Total (%)		
- 4. Execute calculation			
4. Execute calculation		Get results	1

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

Define mode of calculation	
Calculate Single Set of Data	in Managartan In Carrow Ware
C Compare Two Sets of Data for Chang C Compare Two Sets of Data for Chang	· ·
C Compare 1 Wo Sets or Data for Chang	Jes in Detailed Compositions
2. Specify type of result	
Calculations for Theoretical Yield	•
Cell composition Carbon source Electro	n acceptor Energy generation & other parameters
C Glucose C C02 Electron Donor for C02 C Photosynthesis (H20 → 02)	
Electron Donor for CO2	103)
Electron Donor for CO2 Photosynthesis (H20> O2)	103) ℃ Fe[2+) -> Fe[3+]
Electron Donor for C02 Photosynthesis (H20> 02) Nitrification (NH3> HN02> HN	
Electron Donor for CO2 Photosynthesis (H2D> O2) Nitrification (NH3> HNO2> HN S (-2)> S	○ Fe(2+)> Fe(3+)
Electron Donor for CO2	C Fe(2+) → Fe(3+) C Metal ions
Electron Donor for C02	C Fe(2+) -> Fe(3+) C Metal ions Metal

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

🖷, Input			×
<u>C</u> alculator <u>P</u> roblem 9	Sets <u>H</u> elp		
⊢1. Define mode of c	alculation		
Calculate Sir	ngle Set of Data		
	-	hanges in Marcomolecular Compositions	
		hanges in Detailed Compositions	
2. Specify type of re	esult		
Calculations	for Theoretical Yield		-
- 3. Input parameters			
Basis of Calculatio	on:1 g cell		
Cell composition	Carbon source	ectron acceptor Energy generation & other	parameters
Electron Acce	ptor		
O2> H2	20		
- Nitrificatio	on + Denitrification		
(NH3>	HN02> HN03>	N2)	
C S → S(2)	-)	Fe(3+)> Fe(2+)	
🔿 SO4(2-) -	-> S(2-)	C Metal ions	
🔿 SeO3(2-)	> Se(2-)	Metal	м
○ H+> H2	2	Oxidized Charge	4
		Reduced Charge	1
⊢ 4. Execute calculat	ion		
		Get r	results
💐 Input			×
Salculator Problem S	Sets <u>H</u> elp	_	×
			×
<u>Calculator</u> Problem 9	alculation		×
Calculator Problem S	alculation	hanges in Marcomolecular Compositions	×
Calculator Problem S	ngle Set of Data	hanges in Marcomolecular Compositions hanges in Detailed Compositions	×
Calculator Problem S	ngle Set of Data		X
Calculator Problem S	ngle Set of Data vo Sets of Data for C vo Sets of Data for C		×
Calculator Problem S 1. Define mode of c Calculate Sir Compare Tw. Compare Tw. 2. Specify type of re	ngle Set of Data vo Sets of Data for C vo Sets of Data for C		×
Calculator Problem 5	ngle Set of Data ro Sets of Data for C ro Sets of Data for C so Sets of Data for C esult		×
Calculator Problem 3	ngle Set of Data no Sets of Data for C vo Sets of Data for C soult for Theoretical Yield		×
Calculator Problem S 1. Define mode of c Calculate Sir Compare Tw Compare Tw Compare Tw Calculations 3. Input parameters Basis of Calculation	ngle Set of Data vo Sets of Data for C vo Sets of Data for C esult for Theoretical Yield	hanges in Detailed Compositions	
Calculator Problem S 1. Define mode of c Calculate Sir Compare Tw Compare Tw Compare Tw Calculations 3. Input parameters Basis of Calculation	ngle Set of Data vo Sets of Data for C vo Sets of Data for C esult for Theoretical Yield		
Calculator Problem 9 1. Define mode of c Calculate Sin Compare Tw Compare Tw Compare Tw 2. Specify type of re Calculations 3. Input parameters Basis of Calculation Cell composition	ngle Set of Data vo Sets of Data for C vo Sets of Data for C esult for Theoretical Yield	hanges in Detailed Compositions	
Calculator Problem 9 1. Define mode of c Calculate Sin Compare Tw Compare Tw Compare Tw 2. Specify type of re Calculations 3. Input parameters Basis of Calculation Cell composition	alculation ngle Set of Data vo Sets of Data for C vo Sets of Data for C sult for Theoretical Yield on: 1 g cell Carbon source El	hanges in Detailed Compositions	
Calculator Problem 5	alculation ngle Set of Data for Sets of Data for C so Sets of Data for C soult for Theoretical Yield for Theoretical Yield carbon source El lergy Generation Pro	hanges in Detailed Compositions	
Calculator Problem S 1. Define mode of c Calculate Sir Compare Tw Compare Tw 2. Specify type of re Calculations 3. Input parameters Basis of Calculation Cell composition Set 1	alculation	hanges in Detailed Compositions	
Calculator Problem S 1. Define mode of c Calculate Sir Compare Tw Compare Tw 2. Specify type of re Calculations 3. Input parameters Basis of Calculation Cell composition Set 1 0.00	alculation	hanges in Detailed Compositions ectron acceptor Energy generation & other cess D Ratio (mol ATP / mol NADH)	
Calculator Problem S 1. Define mode of c Calculate Sir Compare Tw Compare Tw 2. Specify type of re Calculations 3. Input parameters Basis of Calculation Cell composition Set 1 0.00	alculation ngle Set of Data for C vo Sets of Data for C vo Sets of Data for C esult for Theoretical Yield carbon source EI ergy Generation Pro A1 Su	hanges in Detailed Compositions ectron acceptor Energy generation & other cess D Ratio (mol ATP / mol NADH) 'P Efficiency (%)	
Calculator Problem 5 1. Define mode of c Calculate Sin Compare Tw Compare Tw Calculations 3. Input parameters Basis of Calculation Cell composition Set 1 0.00 15.00 0.00	alculation ngle Set of Data for C vo Sets of Data for C vo Sets of Data for C sult for Theoretical Yield Carbon source El ergy Generation Pro A1 St Us	hanges in Detailed Compositions ectron acceptor Energy generation & other cess D Ratio (mol ATP / mol NADH) 'P Efficiency (%) Igar used in energy generation process	
Calculator Problem 5 1. Define mode of c Calculate Sin Compare Tw Compare Tw Calculations 3. Input parameters Basis of Calculatic Cell composition Set 1 0.00 0	alculation ngle Set of Data for C vo Sets of Data for C vo Sets of Data for C sult for Theoretical Yield Carbon source El ergy Generation Pro A1 Su Us	hanges in Detailed Compositions ectron acceptor Energy generation & other cess P Ratio (mol ATP / mol NADH) P Efficiency (%) Igar used in energy generation process sed in PPP (%)	
Calculator Problem 5 1. Define mode of c Calculate Sin Compare Tw Compare Tw Calculations 3. Input parameters Basis of Calculation Cell composition Set 1 0.00 15.00 0.0	alculation ngle Set of Data for C vo Sets of Data for C vo Sets of Data for C sult for Theoretical Yield Carbon source El ergy Generation Pro A1 Su Us	hanges in Detailed Compositions ectron acceptor Energy generation & other cess P Ratio (mol ATP / mol NADH) 'P Efficiency (%) igar used in energy generation process sed in PPP (%) sed in TCA cycle (%)	
Calculator Problem 5 1. Define mode of c Calculate Sin Compare Tw Compare Tw Calculations 3. Input parameters Basis of Calculatic Cell composition Set 1 0.00 0	alculation	hanges in Detailed Compositions ectron acceptor Energy generation & other cess P Ratio (mol ATP / mol NADH) 'P Efficiency (%) igar used in energy generation process sed in PPP (%) sed in TCA cycle (%)	
Calculator Problem 5 1. Define mode of c Calculate Sin Compare Tw Compare Tw Calculations 3. Input parameters Basis of Calculatic Cell composition Set 1 0.00 0	alculation	hanges in Detailed Compositions ectron acceptor Energy generation & other cess Platic (mol ATP / mol NADH) PEfficiency (%) gar used in energy generation process ued in PPP (%) ued in TCA cycle (%) sed in Fermentation (%) perimental Yield, Y (g biomass / g sugar)	
Calculator Problem 5 1. Define mode of c Calculate Sin Compare Tw Compare Tw Calculations 3. Input parameters Basis of Calculatic Cell composition Set 1 0.00 0	alculation	hanges in Detailed Compositions ectron acceptor Energy generation & other cess Platio (mol ATP / mol NADH) PEfficiency (%) Igar used in energy generation process sed in PPP (%) sed in TCA cycle (%) sed in Fermentation (%)	
Calculator Problem 5 1. Define mode of c Calculate Sin Compare Tw Compare Tw Calculations 3. Input parameters Basis of Calculatic Cell composition Set 1 0.00 0	alculation	hanges in Detailed Compositions ectron acceptor Energy generation & other cess Platic (mol ATP / mol NADH) PEfficiency (%) gar used in energy generation process ued in PPP (%) ued in TCA cycle (%) sed in Fermentation (%) perimental Yield, Y (g biomass / g sugar)	
Calculator Problem 5 1. Define mode of c Calculate Sin Compare Tw Compare Tw Calculations 3. Input parameters Basis of Calculatic Cell composition Set 1 0.00 0	salculation ngle Set of Data for C vo Sets of Data for C vo Sets of Data for C sult for Theoretical Yield carbon source El ergy Generation Pro PC A1 Su Us Us Va Va	hanges in Detailed Compositions ectron acceptor Energy generation & other cess Platic (mol ATP / mol NADH) PEfficiency (%) gar used in energy generation process ued in PPP (%) ued in TCA cycle (%) sed in Fermentation (%) perimental Yield, Y (g biomass / g sugar)	Perameters
Calculator Problem 5 1. Define mode of c Calculate Sir Compare Tw Compare Tw Calculations 3. Input parameters Basis of Calculation Cell composition Options for En Set 1 0.00 15.00 100.00 100.00	salculation ngle Set of Data for C vo Sets of Data for C vo Sets of Data for C sult for Theoretical Yield carbon source El ergy Generation Pro PC A1 Su Us Us Va Va	hanges in Detailed Compositions ectron acceptor Energy generation & other cess D Ratio (mol ATP / mol NADH) P Efficiency (%) gar used in energy generation process eed in PPP (%) seed in TCA cycle (%) seed in TCA cycle (%) perimental Yield, Y (g biomass / g sugar) ty (g biomass / mol ATP)	

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.

🗟, Input			X
Calculator Problem Sets Help			_
1. Define mode of calculation			
C Calculate Single Set of Da	ta		
Compare Two Sets of Data	a for Changes in Marcon	nolecular Compositions	
C Compare Two Sets of Data	a for Changes in Detaile	d Compositions	
2. Specify type of result			
Calculations for Theoretica	l Yield		-
- 3. Input parameters			
Basis of Calculation: 1 g cell			_
Cell composition Carbon sourc	e Electron acceptor	Energy generation & other paramet	ers
Dry biomass cell composition	18:		וו ר
_Set 1			
0.00 0.00	Protein (%)	Detailed Compositions	
0.00	RNA (%)	Detailed Compositions	
0.00 0.00	DNA (%)	Detailed Compositions	
0.00 0.00	Lipids (%)	Detailed Compositions	
	Phospholipids (%)	Detailed Compositions	
	Cell Wall (%)	Detailed Compositions	
100.00	Ash [%]		
100.00 100.00	Total (%)		
- 4. Execute calculation			
		Compare results	
Highlight values with % change	e larger than 10.00	Compare results	

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

egories Cell Yield and Energetics Find Y _{XS} (g biomass / g substrate) Y _{XS(Ash Free)} (g biomass (ashless) / g substrate) Y _{ATP} (g biomass / mol ATP) Energetics Summary:	0.476 0.462 12.48					Figure 7 "Cell Yie and Ene getics," i cell yield (either
		Catabolism		Ana	abolism	estimate
	Energy Generation (Overall) Process	Intermediate Production Process	Polymer Formation / Utilisation	Monomer Production Process	Polymerization Process	or given, Y _{X/ATP}
ATP Production (mmol ATP / g biomass):	1.0869E+01	4.1666E+00	0.0000E+00	-6.8364E+00	-1.0511E+01	amount of ATP
NADH + H ⁺ Production (mmol NADH / g biomass):	3.1482E+01	2.3108E+01	0.0000E+00	-8.8569E+00		generate directly
ATP Balancing Calculation: Catabolic ATP Production (mmol ATP / g biomass): Net NAD(H) Production (mmol NADH / g biomass): P/O Ratio (mol ATP / ½ mol O ₂) ATP Produced by Oxidizing NAD(H) (mmol ATP / g biomass): Anabolic ATP Consumption (Cell Material Manufacturing) (mmol ATP / g biomass): Estimated ATP Required for Cellular Functions (mmol ATP / g biomass): Estimated ATP Required ATP Cellidar Functions	1.5035E+01 4.5732E+01 2.20 1.0061E+02 -1.7347E+01 -9.8299E+01 15.00%					from rea tions or oxidativ phospho ylation o sum- marized

_ _ ×	
+01	Figure 8. "Fate of Glucose," glucose directly linked with bio- synthesis or energy genera- tion process is analyzed.

Polymerization Process

-1.0511E+01

	0.476				
Y _{XS(Ash Free)} (g biomass (ashless) / g substrate)	0.462				
Y _{ATP} (g biomass / mol ATP)	12.48				
Energetics Summary:					
		Catabolism			abolism
	Energy Generation (Overall) Process	Intermediate Production Process	Polymer Formation / Utilisation	Monomer Production Process	Polym Pro
ATP Production (mmol ATP / g biomass):	1.0869E+01	4.1666E+00	0.0000E+00	-6.8364E+00	
NADH + H ⁺ Production (mmol NADH / g biomass):	3.1482E+01	2.3108E+01	0.0000E+00	-8.8569E+00	
Catabolic ATP Production (mmol ATP / g biomass): Net NAD(H) Production (mmol NADH / g biomass): P/O Ratio (mol ATP / ½ mol O ₂)	4.5732E+01				
2					
ATP Produced by Oxidizing NAD(H) (mmol ATP / g biomass): Anabolic ATP Consumption (Cell Material Manufacturing)	1.0061E+02 -1.7347E+01				
ATP Produced by Oxidizing NAD() (mmol ATP / g biomass): Anabolic ATP Consumption	1.0061E+02 -1.7347E+01 -9.8299E+01				

🛋 Results

Categories Cell Yield and Energetics

-

Find

🖷, Results

Categories Composition Summary

•

Find

Figure 9. Al detailed biomas compositions such as amine acid, etc., are summarized in "composition summary.

0	Dry Biomass Compositions					
			Weight %	I		
	Marcomolecule Compositions				-	
	Protein		39.00		-	
	RNA DNA		11.00		-	
	Lipids		3.00		-	
	Phospholipids		5.00		1	
	Cell Wall		38.00		1	
	Polymer		0.00		1	
	Polyphosphate		0.00			
	Ash		3.00			
	Total		100.00			
	Total (Ashless):		97.00			
1	Detailed Cell Composition (Base Protein Weight of Protein among Cell:	ed on cell compositi 0.3900	on of Before Polymer ((g protein / g biomass)	·		
	weight of Floten among Cen.	0.5500	(g protein / g biomass)			
	Protein	Weight (g acid / g biomass)	Weight Ratio of Amino Acids among Protein	Molecular Weight	Mole (mol acid / g biomass)	Mole Ratio o Acids among
	Amino Acids (Total)	0.3900	100.00%	N/A	3.6358E-03	100.00%
	Alanine (Ala)	0.0341	8.75%	89	4.8063E-04	13.22%
	Arginine (Arg)	0.0234	5.99%	174	1.4975E-04	4.12%
	Asparagine (Asn)	0.0112	2.88%	132	9.8526E-05	2.71%
	Aspartate (Asp)	0.0331	8.48%	133	2.8758E-04	7.91%
	Cysteine (Cys)	0.0007	0.17%	121	6.4369E-06	0.18%
	Glutamate (Glu)	0.0371	9.50%	147	2.8721E-04	7.90%
	Glutamine (Gln)	0.0129	3.30%	146	1.0055E-04	2.77% 8.79%
		0.0400				
reaction of	Glycine (Gly)	0.0182	4.67%	75 In F F er Utilisation Energy Balance Calvin Cy	3.1953E-04	4 700/
	it i = 4: 4: = - 7: 1: = 1 istais		0.000/			
	detais Setais F6P from Carbon Source		ectron Acceptors Polymer Formation Polym	e Utilization Energy Balance Calvin Cy NADPH -	R PPP for Biot	synthesis 6-P-Glucon
	in tion () () () () () () () () () (Biosynthesis 2 Energy Generation El	ectron Acceptors Polymer Formation Polym	er Utilisation Energy Balance Calvin Cys NADPH - 9.6941E-	R PPP for Biot	
		Biogenthesis 2 Energy Generation El	ectron Acceptors Polymer Formation Polymer	e Utilization Energy Balance Calvin Dy	R PPP for Biot	synthesis 6-P-Glucon
		Biographics 2 Energy Generation El	ectron Acceptors Polymer Formation Polymer	H + F F H Utilization Energy Balance Calvin Cy NADPH - 9.694 TE-	36 36 24	synthesis 6-P-Glucon
		Biogenthesis 2 Energy Generation El	ectron Acceptors Polymer Formation Polymer	H + F F H Utilization Energy Balance Calvin Cy NADPH - 9.694 TE-	R PPP for Biot	synthesis 6-P-Glucon
		Biogenthesis 2 Energy Generation El ATP 4.6660E-05 ADP 4.6660E-05 G6P	ectron Acceptors Polymer Formation Polymer 0E-05 Pi 0.0000E+00	er Utilisation Energy Balance Calvin Da NADPH 9 6541E-04 H ₂ O	PPP for Bior PPP for Bior CO ₂ 9.6541E-04	rynthesis 6.P. Glucon 9.694 IE-04 Ribulos - 5
in DNA F	tick dia	Biogenhesis 2 Energy Generator El	ectron Acceptors Polymer Formation Polymer 0E-05 Pi 0.0000E+00	e Utilization Energy Balance Calvin Dy NADPH 9 6541E- 9 6541E-04	PPP for Bior PPP for Bior CO ₂ 9.6541E-04	synthesis 6.P.Glucon 9.6941E.04
in DNA.F	tick dia	Biogenthesis 2 Energy Generation El ATP 4.6660E-05 ADP 4.6660E-05 G6P	ectron Acceptors Polymer Formation Polymer 0E-05 Pi 0.0000E+00	e Utilization Energy Balance Calvin Cy NADP+ 9.6541E-04 H ₂ O 9.6541E-14 H ₂ O 9.6541E-14 Glucose di P	PPP for Bior PPP for Bior CO ₂ 9.6541E-04	rynthesis 6.P. Glucon 9.694 IE-04 Ribulos - 5
in DNA F	tick dia	Biogenthesis 2 Energy Generation El ATP 4.6660E-05 ADP 4.6660E-05 G6P	ectron Acceptors Polymer Formation Polymer 0E-05 Pi 0.0000E+00	NADP+ 9.6341E-04 9.6341E-04	CO ₂ 964 04 04 04 04 04	ynthesis 6.P.Glucon 9.6941E.04 Ribulose.5 9.6941E.04 R5P
in DNA F	tick dia	Biogenthesis 2 Energy Generation El ATP 4.6660E-05 ADP 4.6660E-05 G6P	ectron Acceptors Polymer Formation Polymer 0E-05 Pi 0.0000E+00	e Utilization Energy Balance Calvin Cy NADP+ 9.6541E-04 H ₂ O 9.6541E-14 H ₂ O 9.6541E-14 Glucose di P	54 CO ₂ 9.6341E-04	rynthesis E.P. Glucon 9.6941E-04 Ribulose 5 9.6941E-04
in DNA F	tick dia	Biogenthesis 2 Energy Generation El ATP 4.6660E-05 ADP 4.6660E-05 G6P	ectron Acceptors Polymer Formation Polymer 0E-05 Pi 0.0000E+00	Arr NADPH 9.6941E-04 H ₂ O	CO ₂ 964 04 04 04 04 04	ynthesis 6.P.Glucon 9.6941E.04 Ribulose.5 9.6941E.04 R5P
in DNA F	tick dia	Biogenthesis 2 Energy Generation El ATP 4.6660E-05 ADP 4.6660E-05 G6P	ectron Acceptors Polymer Formation Polymer 0E-05 Pi 0.0000E+00	et Utilization Energy Balance Calvin Cy NADP+ 9 6541E- 4	BPP for Bior BPP for Bior CO ₂ 9.6941E-04 04 CO ₂ 9.6941E-04 04 CO ₂ 9.6941E-04 04	rynthesis 6.P.Glucon 9.6941E-04 Ribulose 5 9.6941E-04 R5P 2.4833E-04
in DNA F	tick dia	Biogenthesis 2 Energy Generation El ATP 4.6660E-05 ADP 4.6660E-05 G6P	ectron Acceptors Polymer Formation Polymer 0E-05 Pi 0.0000E+00	et Utilization Energy Balance Calvin Cy NADP+ 9 6541E- 4	CO ₂ 9.6841E-04 04	ynthesis 6.P.Glucon 9.6941E.04 Ribulose.5 9.6941E.04 R5P 2.4839E.04
in DNA F	tick dia	Biogenthesis 2 Energy Generation El ATP 4.6660E-05 ADP 4.6660E-05 G6P	ectron Acceptors Polymer Formation Polymer 0E-05 Pi 0.0000E+00	et Utilization Energy Balance Calvin Cy NADP+ 9 6541E- 4	BPP for Bior BPP for Bior CO ₂ 9.6941E-04 04 CO ₂ 9.6941E-04 04 CO ₂ 9.6941E-04 04	rynthesis 6.P.Glucon 9.6941E-04 Ribulose 5 9.6941E-04 R5P 2.4833E-04
in DNA F	tional: F6P from Carbon Source F6P from Carbon Source 1.1675E-02 Phosphoryl Compound F6P F6P F6P	Biogenthesis 2 Energy Generation El ATP 4.6660E-05 ADP 4.6660E-05 G6P	ectron Acceptors Polymer Formation Polymer 0E-05 Pi 0.0000E+00	Image: Press Balance Calvin Cy NADP* 9.6541E- 9.6541E-04 H20 9.6541E-04 H20 9.6541E-03 Silucose.di.P 2.3457E-03 Glucose.di.P 2.3457E-03 Glucose.di.P	2 0000 00 2 00 0	ynthesis 6.P.Glucon 9.6941E.04 Ribulos 5. 9.6941E.04 RSP 2.4833E.04 S7P 2.4833E.04 E4P
in DNA F	tion tions of the second seco	Biogenthesis 2 Energy Generation El ATP 4.6660E-05 ADP 4.6660E-05 G6P	ectron Acceptors Polymer Formation Polymer 0E-05 Pi 0.0000E+00	Image: Program (International International Internatione Internatina Internatione International International Internati	H* PPP for Bior CO ₂ 9.6941E-04 04 CO ₂ 9.6941E-04 04 CO ₂ 9.6941E-04 04	rynthesis 6.P.Glucon 9.6941E.04 Ribulose 5 9.6941E.04 RSP 2.4833E.04
5P 5P	H Linki Jine A Hinki S Half Linki Cat Wall Detailed Compositions Biosynthesis 1 F6P from Carbon Source 1.1675E-02 Phosphoryl Compound F6P 1.1675E-02	Biogenthesis 2 Energy Generation El ATP 4.6660E-05 ADP 4.6660E-05 G6P	ectron Acceptors Polymer Formation Polymer 0E-05 Pi 0.0000E+00	e Utilization Energy Balance Calvin Cy ar Utilization Energy Balance Calvin Cy 9 6941E-04 H ₂ O 9 6941E-0 Giucose di P 2.3457E-03 G1P 2.3457E-03	2 0000 00 2 00 0	ynthesis 6.P.Glucon 9.6941E.04 Ribulose.5 9.6941E.04 RSP 2.4839E.04 E4P 2.4839E.04
5 5 7478E-04	H Linki Jine A Hinki S Half Linki Cat Wall Detailed Compositions Biosynthesis 1 F6P from Carbon Source 1.1675E-02 Phosphoryl Compound F6P 1.1675E-02	Biogenthesis 2 Energy Generation El ATP 4.6660E-05 ADP 4.6660E-05 G6P	ectron Acceptors Polymer Formation Polymer 0E-05 Pi 0.0000E+00	Image: Press Balance Calvin Cy NADP* 9.6541E- 9.6541E-04 H20 9.6541E-04 H20 9.6541E-03 Silucose.di.P 2.3457E-03 Glucose.di.P 2.3457E-03 Glucose.di.P	2 0000 00 2 00 0	rynthesis 6.P.Glucon 9.6941E.04 Ribulose 5 9.6941E.04 R5P 2.4839E.04 E4P 2.4839E.04 G3P
5P 5P	H Linki Jine A Hinki S Half Linki Cat Wall Detailed Compositions Biosynthesis 1 F6P from Carbon Source 1.1675E-02 Phosphoryl Compound F6P 1.1675E-02	Biogenthesis 2 Energy Generation El ATP 4.6660E-05 ADP 4.6660E-05 G6P	ectron Acceptors Polymer Formation Polymer 0E-05 Pi 0.0000E+00	e Utilization Energy Balance Calvin Cy ar Utilization Energy Balance Calvin Cy 9 6941E-04 H ₂ O 9 6941E-0 Giucose di P 2.3457E-03 G1P 2.3457E-03	2 0000 00 2 00 0	rynthesis 6.P.Glucon 9.6941E.04 Ribulose 5 9.6941E.04 R5P 2.4839E.04 E4P 2.4839E.04 G3P
5P 5P	tion tion of the second secon	Biogenthesis 2 Energy Generation El ATP 4.6660E-05 ADP 4.6660E-05 G6P	ectron Acceptors Polymer Formation Polymer 0E-05 Pi 0.0000E+00	e Utilization Energy Balance Calvin Cy ar Utilization Energy Balance Calvin Cy 9 6941E-04 H ₂ O 9 6941E-0 Giucose di P 2.3457E-03 G1P 2.3457E-03	24335E-04 F6P	rynthesis 6.P.Glucon 9.6941E.04 Ribulose 5 9.6941E.04 R5P 2.4839E.04 E4P 2.4839E.04 G3P
5P 5P	H Linki Jine A Hinki S Half Lipids Cat Wall Detailed Compositions Biosynthesis 1 F6P from Carbon Source 1.1675E-02 Phosphoryl Compound F6P 1.1675E-02 0 F6P from G6P and PPP	Biogenthesis 2 Energy Generation El ATP 4.6660E-05 ADP 4.6660E-05 G6P	ectron Acceptors Polymer Formation Polymer 0E-05 Pi 0.0000E+00	e Utilization Energy Balance Calvin Cy ar Utilization Energy Balance Calvin Cy 9 6941E-04 H ₂ O 9 6941E-0 Giucose di P 2.3457E-03 G1P 2.3457E-03	24335E-04 F6P	rynthesis 6.P.Glucon 9.6941E.04 Ribulose 5 9.6941E.04 R5P 2.4839E.04 E4P 2.4839E.04 G3P
5P 5P 59 59 59 59 59 59 59 59 59 59 50 50 50 50 50 50 50 50 50 50 50 50 50	tion tion of the second secon	Biogenthesis 2 Energy Generation El ATP 4.6660E-05 ADP 4.6660E-05 G6P	ection Acceptors Polymer Formation Polymer Fise JE-05 Pi 0.0000E+00 JE-03 GEP (From Glucose	e Utilization Energy Balance Cavin Dy NADP+ 9.6941E-04 9.6941E-04 9.6941E-04 2.3457E-03 GIP 2.3457E-03 GIP 2.3457E-03 Holysaccharides	2 4839E-04 F6P 2 4839E-04 CO ₂ 9 6841E-04 04 CO ₂ 9 6841E-04 04 CO ₂ 9 6841E-04 04 CO ₂ 9 6841E-04 04 CO ₂ 9 6841E-04 CO ₂ 0 7 0 7 0 7 0 7 0 7 0 7 0 7 0 7	ynthesis 6.P.Glucon 9.6941E.04 Ribulose.5 9.6541E.04 RSP 2.4839E.04 STP 2.4839E.04 E4P 2.4839E.04
5P 5P	tion tions of the second seco	Biogenthesis 2 Energy Generation El ATP 4.6660E-05 ADP 4.6660E-05 G6P	ection Acceptors Polymer Formation Polymer Pie-05 Pi 0.0000E+00 3E-03 G6P (From Glucose 3.7478E-04	Image: Press Balance Cavin Cy NADP* 9.6941E-04 9.6941E-04 Hg.0 9.6941E-04 Hg.0 9.6941E-03 Side of the comparison of the	CO2 9.6541E-04 04 CO2 9.6541E-04 04 CO2 9.6541E-04 04 CO2 9.6541E-04 04 CO2 9.6541E-04 CO2 9.6541E-04 CO2 9.6541E-04 CO2 9.6541E-04	ynthesis 6.P.Glucon 9.6941E.04 Ribulose.5 9.6941E.04 FSP 2.4839E.04 S7P 2.4839E.04 E4P 2.4839E.04 G3P 0.0000E+00
n DNA f	tion tions of the second seco	Biogenthesis 2 Energy Generation El ATP 4.6660E-05 ADP 4.6660E-05 G6P	ection Acceptors Polymer Formation Polymer Fise JE-05 Pi 0.0000E+00 JE-03 GEP (From Glucose	e Utilization Energy Balance Cavin Dy NADP+ 9.6541E-04 9.6541E-04 2.3457E-03 GIP 2.3457E-03 GIP 2.3457E-03 Holysaccharides	CO2 9.6541E-04 04 CO2 9.6541E-04 04 CO2 9.6541E-04 04 CO2 9.6541E-04 04 CO2 9.6541E-04 CO2 9.6541E-04 CO2 9.6541E-04 CO2 9.6541E-04 CO2 9.6541E-04 CO2 9.6541E-04 CO2 9.6541E-04 CO2 9.6541E-04 CO2 9.6541E-04 CO2 9.6541E-04 CO2 9.6541E-04 CO2 9.6541E-04 CO2 9.6541E-04 CO2 9.6541E-04 CO2 9.6541E-04 CO2 9.6541E-04 CO2 CO2 9.6541E-04 CO2 CO2 9.6541E-04 CO2 CO2 CO2 CO2 CO2 CO2 CO2 CO2 CO2 CO2	rynthesis 6.P.Glucon 9.6941E.04 Ribulose 5. 9.6941E.04 R5P 2.4839E.04 S7P 2.4839E.04 E4P 2.4839E.04
50 50 50 50 50 56 56 55 56 55 56 55 56 55 50 50 50 50 50 50 50 50 50 50 50 50	time in the second sec	Biogenthesis 2 Energy Generation El ATP 4.6660E-05 ADP 4.6660E-05 G6P	ectron Acceptors Polymer Formation Polymer See JE-05 Pi 0.0000E+00 JE-03 JE-03 GEP (From Glucose 3.7478E-04 G6P (From PPP)	Image: Pregr Balance Cavin Cy NADP+ 9.6341E- 9.6341E-04 H ₂ O 9.6341E-04 H ₂ O 9.6341E-03 Siducose. di-P 2.3457E-03 Polysaccharides	CO2 9.6541E-04 04 CO2 9.6541E-04 04 CO2 9.6541E-04 04 CO2 9.6541E-04 04 CO2 9.6541E-04 CO2 9.6541E-04 CO2 9.6541E-04 CO2 9.6541E-04 CO2 9.6541E-04 CO2 9.6541E-04 CO2 9.6541E-04 CO2 9.6541E-04 CO2 9.6541E-04 CO2 9.6541E-04 CO2 9.6541E-04 CO2 9.6541E-04 CO2 9.6541E-04 CO2 9.6541E-04 CO2 9.6541E-04 CO2 9.6541E-04 CO2 CO2 9.6541E-04 CO2 CO2 9.6541E-04 CO2 CO2 CO2 CO2 CO2 CO2 CO2 CO2 CO2 CO2	rynthesis 6.P.Glucon 9.6941E.04 9.6941E.04 9.6941E.04 8.5P 2.4839E.04 4.4839E.04 6.3P 2.4839E.04 4.4839E.04 6.3P 6.30000E+00 H ₂ O
5P 5P 50000E+0 7P 7P 7P 7P 7P 7P	time in the second sec	Biogenthesis 2 Energy Generation El ATP 4.6660E-05 ADP 4.6660E-05 G6P	ectron Acceptors Polymer Formation Polymer See JE-05 Pi 0.0000E+00 JE-03 JE-03 GEP (From Glucose 3.7478E-04 G6P (From PPP)	Image: Pregr Balance Cavin Cy NADP+ 9.6341E- 9.6341E-04 H ₂ O 9.6341E-04 H ₂ O 9.6341E-03 Siducose. di-P 2.3457E-03 Polysaccharides	CO ₂ 9.6941E-04 04 CO ₂ 9.6941E-04 04 CO ₂ 9.6941E-04 04 CO ₂ 9.6941E-04 04 CO ₂ 9.6941E-04 CO ₂ 9.7451E-04 CO ₂ 9.7551E-04 CO ₂ 9.755	rynthesis 6.P.Glucon 9.6941E.04 9.6941E.04 9.6941E.04 8.5P 2.4839E.04 4.4839E.04 6.3P 2.4839E.04 4.4839E.04 6.3P 6.30000E+00 H ₂ O

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

Chemical Engineering Education

_ 🗆 ×

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 / gbiomass 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 +

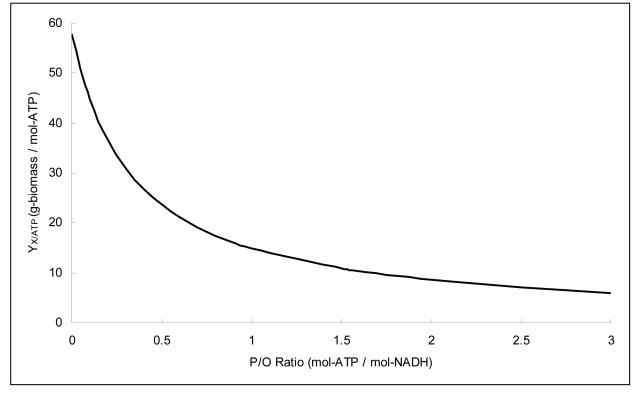


Figure 11. Relationship between $Y_{X/ATP}$ and P/O ratio.

P/O x 50.1 mmol-ATP) / 1.07 g-glucose. And $Y_{X/ATP} = 1$ gbiomass / 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.

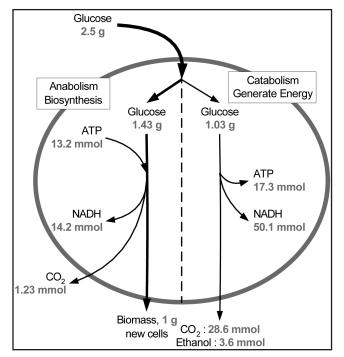


Figure 12. Glucose used for biosynthesis and energy generation purposes, drawn based on Metstoich results.

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.

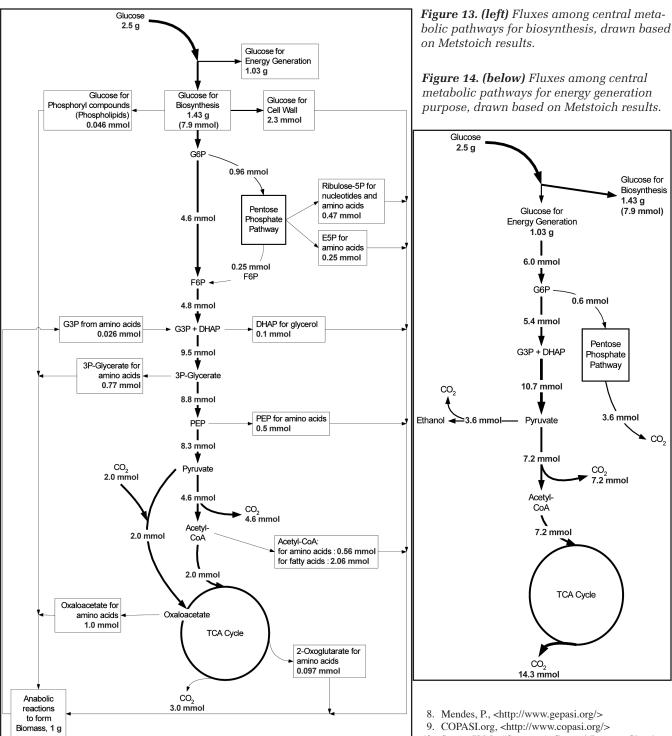
The package has been well received by both academic experts in biochemical engineering and undergraduate chemical engineering and biochemistry students at HKUST.

ACKNOWLEDGMENT

The authors would like to acknowledge the financial support (Project : HKUST 00409E) of the Center For Enhanced Learning & Teaching (CELT) as well as their technical participation in the project.

REFERENCES

- Nielsen, J., "Metabolic Engineering: Techniques for Analysis of Targets for Genetic Manipulations," *Biotech. Bioeng.*, 58, 125-132 (1998)
- Olsson, L., and J. Nielsen, "The Role of Metabolic Engineering in the Improvement of Saccharomyces cerevisiae: Utilization of Industrial Media," Enzyme Microb. Technol., 26, 785-792 (2002)
- 3. Stephanopoulos, G.N., A.A. Aristidou, and J. Nielsen, Metabolic



Engineering, Principles, and Methodologies, Academic Press (1998)

- 4. Lee, S.Y., and E.T. Papoutsakis. Ed., Metabolic Engineering, Marcel Dekker, Inc. (1999)
- Varma, A., and B.O. Palsson, "Metabolic Flux Balancing: Basic Concepts, Scientific and Practical Use," *Bio/technology*, 12, 994-998 (1994)
- Weichert, W., "Modeling and Simulation: Tools for Metabolic Engineering," J. Biotech., 94, 37-63 (2002)
- Mendes, P., "Biochemistry by Numbers: Simulation of Biochemical Pathways with Gepasi 3," *Trends Biochem. Sci.*, 22, 361-363 (1997)

- Sauro, H.M., "Scamp: A General-Purpose Simulator and Metabolic Control Analysis Program," *Comput. Appl. Biosci.*, 9, 441-450 (1993)
- Tomita, M., K. Hashimoto, K. Takahashi, T.S. Shimizu, Y. Matsuzaki, F. Muyoshi, K. Saito, S. Tanida, K. Yugi, J.C. Venter, and C.A. Hutchison III, "E-CELL: Software Environment for Whole-Cell Simulation," *Bioinformatics*, **15**, 72-84 (1999)
- Ehlde, M., and G. Zacchi, "Mist: A User-friendly Metabolic Simulator," *Comp. App. Biosci. (CABIOS)*, 11, 201-207 (1995)

- Olivier, B., and J. Snoep, <http://jjj.biochem.sun.ac.za/> (2002-2003)
- Barshop, B.A., R.F. Wrenn, and C. Frieden, "Analysis of Numerical Methods for Computer Simulation of Kinetic Processes: Development of KINSIM – A Flexible, Portable System," *Anal. Biochem.*, 130, 134-145 (1983)
- Dang, Q., and C. Frieden, "New PC Versions of the Kinetic-Simulation and Fitting Programs, KINSIM and FITSIM," *Trends Biochem. Sci.*, 22, 317 (1997)
- Klamt, S., J. Stelling, M. Ginkel, and E.D. Gilles, "FluxAnalyzer: Exploring Structure, Pathways, and Flux Distributions in Metabolic Networks on Interactive Flux Maps," *Bioinformatics*, 19, 261-269 (2003)
- Klamt, S., S. Schuster, and E.D. Gilles, "Calculability Analysis in Underdetermined Metabolic Networks Illustrated by a Model of the Central Metabolism in Purple Nonsulfur Bacteria," *Biotech. Bioeng.*, 77, 734-751 (2002)
- Neidhardt, F.C., J.L. Ingraham, and M. Schaechter, *Physiology of the* Bacterial Cell – A Molecular Approach, Sinauer Associates, (1990)
- 19. CellDesigner.org, <http://celldesigner.org/>
- 20. Cellerator.info, <http://www.cellerator.info/>
- 21. InNetics.com, <http://innetics.com/>
- 22. JigCell Project, <http://jigcell.biol.vt.edu/>
- 23. Wong, K.W., J.P. Barford, and J.F. Porter, "Understanding the Practi-

cal Consequences of Metabolic Interactions — A Software Package for Teaching and Research," *Computer Applications in Biotechnology*, 9th International Symposium, Nancy, France (2004)

- 24. Wong, K.W., J.P. Barford, and J.F. Porter, "Understanding the Practical Consequences of Metabolic Interactions—A Software Package for Teaching and Research," *Proceedings* of the Second Teaching & Learning Symposium, The Hong Kong University of Science & Technology, Hong Kong (2004)
- 25. Oura, E., "The Effect of Aeration on the Growth Energetics and Biochemical Composition of Baker's Yeast" with appendix: "Reactions Leading to the Formation of Yeast Cell Material from Glucose and Ethanol," Ph.D. Thesis, Helsinki University, Helsinki, Finland, (1972)
- Sanderson, C.S., J.P. Barford, and G.W. Barton, "A Structured Dynamic Model for Animal Cell Culture Systems," *Biochem. Eng. Journal*, 3, 203-211 (1999)
- Sanderson, C.S., J.P. Barford, G.W. Barton, T.K. Wong, and S. Reid, "A Structured Dynamic Model for Animal Cell Culture Systems: Application to Baculovirus / Insect Cell Systems," *Biochem. Eng. Journal*, 3, 219-229 (1999)
- Sanderson, C.S., J.D. Jang, J.P. Barford, and G.W. Barton, "A Structured Dynamic Model for Animal Cell Culture Systems: Application to Murine Hybridoma," *Biochem. Eng. Journal*, 3, 213-218 (1999)