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

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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, $Y_{ATP/X}$ 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): $h = \frac{ATP_{Consumed, Anabolism}}{ATP_{Consumed, Total}}$. 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. $Y_{ATP/X}$ 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.

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Input

Calculator Problem Sets Help

1. Define mode of calculation
☒ Calculate single set of data ☐ Compare two sets of data

2. Specify type of result
 Calculations: for Theoretical Yield

3. Input parameters
 Basis of Calculation: 1 g cell
 Dry biomass cell compositions:

Set 1

0.00	Protein (%)
0.00	RNA (%)
0.00	DNA (%)
0.00	Lipids (%)
0.00	Phospholipids (%)
0.00	Cell Wall (%)
100.00	Ash (%)
100.00	Total (%)

0.00	PO Ratio (mol ATP / 1/2 mol O ₂)
15.00	ATP Efficiency (%)
0.00	Glucose Used in Catabolism for PPP (%)
0.00	Glucose Used in Catabolism for TCA cycle (%)
100.00	Glucose Used in Catabolism for Fermentation (%)
	Experimental Yield, Y (g biomass / g glucose)
	Yatp (g biomass / mol ATP)

4. Execute calculation
 Get results

Figure 3. Input interface (Blank)

Input

Calculator Problem Sets Help

1. Define mode of calculation
☒ Calculate single set of data ☐ Compare two sets of data

2. Specify type of result
 Calculations: for Theoretical Yield

3. Input parameters
 Basis of Calculation: 1 g cell
 Dry biomass cell compositions:

Set 1

40.00	Protein (%)
10.00	RNA (%)
8.00	DNA (%)
12.00	Lipids (%)
4.00	Phospholipids (%)
9.00	Cell Wall (%)
17.00	Ash (%)
100.00	Total (%)

2.20	PO Ratio (mol ATP / 1/2 mol O ₂)
15.00	ATP Efficiency (%)
25.00	Glucose Used in Catabolism for PPP (%)
30.00	Glucose Used in Catabolism for TCA cycle (%)
45.00	Glucose Used in Catabolism for Fermentation (%)
	Experimental Yield, Y (g biomass / g glucose)
	Yatp (g biomass / mol ATP)

4. Execute calculation
 Get results

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

Figure 5. Calculation result – ‘Cell Yield and Energetics’

Figure 6 Calculation result – ‘Fate of Glucose’

Figure 7. Problem sets for the trial of METSTOICH

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

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