Metabolic Engineering

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PRINCIPLES AND METHODOLOGIES

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To Our Families

PREFACE

Metabolic engineering is about the analysis and modification of metabolic pathways. The field emerged during the past decade, and powered by techniques from applied molecular biology and reaction engineering, it is becoming a focal point of research activity in biological and biochemical engineering, cell physiology, and applied microbiology. Although the notion of pathway manipulation had been discussed before, the vision of metabolic engineering as defining a discipline in its own right was first suggested by Bailey in 1991. It was embraced soon thereafter by both engineers and life scientists who saw in it the opportunity to capture the potential of sequence and other information generated from genomics research.

We first attempted to convey the excitement and basic concepts of metabolic engineering to our students in a course that was taught at MIT in 1993. The experiment was repeated again in 1995 and 1997, at which time a definite syllabus and tentative set of notes had emerged as a result of these offerings. A similar development occurred at the Technical University of Denmark (DTU), where metabolic engineering has been a central topic in biochemical engineering courses at both the undergraduate and the graduate level. In 1996, a standard one-semester course on metabolic engineering was offered for the first time. The growing interest in metabolic engineering and requests to share the course material led us to the decision to write this book. In so doing, we have tried to formulate a framework of quantitative biochemistry for the analysis of pathways of enzymatic reactions. In this sense, the book reflects a shift of focus from equipment toward single cells, as it concentrates on the elucidation and manipulation of their biochemical

functions. As such, this text can support a graduate or advanced undergraduate course on metabolic engineering to complement current offerings in biochemical engineering.

The book manuscript was used to teach courses on metabolic engineering at MIT and DTU, as well as a summer course at MIT. The material can be covered in a single semester with no prerequisites, although some prior exposure to an introductory biochemistry course is helpful. Assigned readings from biochemistry texts during the first quarter of the semester can complement the first part of the book. Problem sets aiding the understanding of basic concepts will be posted periodically at the web site listed below. Although the focus of this book is on metabolism, the concepts of pathway analysis are broad and as such generally applicable to other types of reaction sequences, including those involved in protein expression and post-translational modification or in signal transduction pathways.

Writing a book on a subject that is still in its formative stage is a challenge that carries with it increased responsibility. For this reason, we set as our goal to define core *principles* central to pathway design and analysis, complemented with specific *methods* derived from recent research. We expect these methods to evolve further and hope that this book will play a role in catalyzing such activity. Software implementing the various methods can be found at the book web site, http://www.cpb.dtu.dk/cpb/metabol.htm, with hyperlinks to other sites where public domain software is available. To facilitate broad interdisciplinary participation, only codes with a minimum of service and user-friendliness have been selected. Furthermore, the mathematical complexity of the book has been kept to an absolute minimum, and background material has been provided wherever possible to assist the less mathematically inclined. We are aware of the challenges of this task and the difficulties in satisfying all segments of the readership spectrum. We encourage readers to continue their review of the book undeterred by any temporary difficulties.

We are indebted to many individuals for their direct contributions or indirect input in planning and executing this project. First, we thank our students for their boundless energy and refreshing creativity, in particular Maria Klapa, for a thorough review of metabolic flux analysis, and Troy Simpson, whose research provided the basis for complex pathway analysis. Also, we thank Martin Bastian Pedersen for drafting many of the figures, and Christian Müller, Susanne Sloth Larsen, Birgitte Karsbøl, and Kristen Nielsen for their help in finalizing the manuscript. We thank our colleagues, in particular Tony Sinskey, for his enthusiasm about the unlimited possibilities of metabolic engineering, and Sue Harrison and Eduardo Agosin for their most constructive comments. Finally, we thank our collaborators and friends,

in particular, Barry Buckland, Bernhard Palsson, John Villadsen, Maish Yarmush, and D. Ramkrishna. Their vision and unwavering support when it mattered meant a lot.

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LIST OF SYMBOLS

Below is a list of the symbols most frequently used throughout this text. The unit specified is the most typically applied unit, in some cases the symbols may have another unit.

a_{cell} Specific surface area of the cells (m^2 (g DW)$^{-1}$)

a Row vector containing weights of individual variables on the objective function in eq. (8.26)

A_i Affinity of the ith reaction (kJ mole^{-1})

c Concentration (mmoles L^{-1})

c_i Concentration of the ith compound (mmoles L^{-1})

c_i^f Concentration of the ith compound in the feed to the bioreactor (mmoles L^{-1})

$C_i^{J_j}$ Flux control coefficient for the ith enzyme on the jth steady state flux J_j

$^*C_i^{J_j}$ Group flux control coefficient for the ith group on the steady state flux J_j

$C_i^{X_j}$ Concentration control coefficient for the ith enzyme on the jth metabolite concentration

$^*C_i^{X_j}$ Group concentration control coefficient for the ith group on the jth metabolite concentration

$\mathbf{C}^J$ Matrix containing flux control coefficients

$\mathbf{C}^X$ Matrix containing concentration control coefficients

d_{mem} Thickness of the cytoplasmic membrane (m)

D Dilution rate (h^{-1})

D_{mem} Diffusion coefficient for membrane diffusion (m^2 s^{-1})

D_i^J Deviation index given by eq. (11.84)

E_i Activity (or concentration) of the ith enzyme

E Elemental composition matrix or matrix containing elasticity coefficients

$\mathbf{E}_c$ Elemental composition matrix for non-measured compounds
$\mathbf{E}_m$ Elemental composition matrix for measured compounds
f Flux amplification factor given by eq. (11.87)
f_{ij} The ratio of flux j to flux i (given by eq. (11.50))
F Volumetric flow rate into the bioreactor (L h^{-1})
F_{out} Volumetric flow rate out of the bioreactor (L h^{-1})
$\mathbf{F}$ Variance-covariance matrix
g_{ij} Stoichiometric coefficient for the ith intracellular metabolite in the jth reaction
G Gibbs function (kJ $mole^{-1}$)
ΔG Gibbs free energy change (kJ $mole^{-1}$)
$\Delta G^{0'}$ Gibbs free energy change with all reactants and products at their standard states (kJ $mole^{-1}$)
$\mathbf{G}$ Matrix containing the stoichiometric coefficients for the intracellular metabolites
$\mathbf{G}_c$ Matrix containing the stoichiometric coefficients for intracellular metabolites in reactions for which fluxes are not measured
$\mathbf{G}_m$ Matrix containing the stoichiometric coefficients for intracellular metabolites in reactions for which fluxes are measured
$\mathbf{G}_{ex}$ Stoichiometric matrix for a metabolic model containing the stoichiometry for all reactions both in a forward and in a reverse direction
h Test function given by eq. (4.29)
$h_{s,i}$ Carbon content in the ith substrate (C-moles $mole^{-1}$)
$h_{p,i}$ Carbon content in the ith metabolic product (C-moles $mole^{-1}$)
H Enthalpy function (kJ $mole^{-1}$)
H_j Thermodynamic function given by eq. (14.29)
$\mathbf{I}$ Identity matrix, *i.e.*, matrix with all diagonal elements being 1 and all other elements being 0
j Flow ratio given by eq. (14.46)
J_i Steady state flux through the ith pathway branch (mmoles $(g\ DW\ h)^{-1}$)
$\mathbf{J}$ Vector of steady state fluxes (mmoles $(g\ DW\ h)^{-1}$)
$\mathbf{J}_{dep}$ Vector of dependent fluxes (mmoles $(g\ DW\ h)^{-1}$)
$\mathbf{J}_{in}$ Vector of independent fluxes (mmoles $(g\ DW\ h)^{-1}$)
k Rate constant (h^{-1})
K The number of intracellular metabolites considered in the analysis
K_{eq} Equilibrium constant
K_{par} Partitioning coefficient between the lipid membrane and the medium (dimensionless)

K_m Michaelis-Menten constant (or saturation constant) (mmoles L^{-1})

K_i Inhibition constant (mmoles L^{-1})

$\mathbf{K}$ Kernel matrix which fulfills eq. (12.7)

L_{ij} Phenomenological coefficients

m_{ATP} ATP requirements for maintenance metabolism (mmoles ATP $(g\,DW\,h)^{-1}$)

M The number of metabolic products considered in the analysis

N The number of substrates considered in the analysis

p Parameters that influence reaction rate (used in eq. (11.5))

P Permeability coefficient (m s^{-1})

P_i The ith metabolic product

$\mathbf{P}$ Variance-covariance matrix of residuals (given by eq. (4.24)) or matrix containing the parameter elasticity coefficients

q The degree of coupling

Q The number of macromolecular pools considered in the analysis

Q_{heat} Heat production associated with biomass growth (kJ (C-moles biomass)$^{-1}$)

r Specific rate (mmoles $(g\,DW\,h)^{-1}$)

r_{ATP} Rate of ATP production (mmoles $(g\,DW\,h)^{-1}$)

r_i Activity amplification factor given by eq. (11.86)

$r_{macro,i}$ Specific rate of formation of the ith macromolecular pool (g $(g\,DW\,h)^{-1}$)

$r_{met,i}$ Specific rate of formation of the ith intracellular metabolite (mmoles $(g\,DW\,h)^{-1}$)

r_p Specific product formation rate (mmoles $(g\,DW\,h)^{-1}$)

r_s Specific substrate uptake rate (mmoles $(g\,DW\,h)^{-1}$)

r_{tran} Specific rate of transport across the cytoplasmic membrane (mmoles $(g\,DW\,h)^{-1}$)

$\mathbf{r}_c$ Vector of non-measured specific rates (mmoles $(g\,DW\,h)^{-1}$)

$\mathbf{r}_m$ Vector of measured specific rates (mmoles $(g\,DW\,h)^{-1}$)

$\mathbf{r}_{macro}$ Vector containing the specific rates of macromolecular formation (g $(g\,DW\,h)^{-1}$)

$\mathbf{r}_{met}$ Vector containing the specific rates of intracellular metabolite formation (mmoles $(g\,DW\,h)^{-1}$)

$\mathbf{r}_p$ Vector containing the specific rates of metabolic product formation (mmoles $(g\,DW\,h)^{-1}$)

$\mathbf{r}_s$ Vector containing the specific rates of substrate uptake (mmoles $(g\,DW\,h)^{-1}$)

R Gas constant (= 0.008314 kJ (K-mole)$^{-1}$)

R_i Activity amplification parameter given by eq. (13.40)

$R_{X_i}^{J_j}$ Response coefficient given by eq. (11.7)

$\mathbf{R}$ Redundancy matrix given by eq. (4.17)

$\mathbf{R}_r$ Reduced redundancy matrix containing independent rows of $\mathbf{R}$

S Entropy function (kJ (K mole)$^{-1}$)

S_i The ith substrate

T Temperature (K)

$\mathbf{T}$ Matrix containing stoichiometric coefficients as specified by eq. (8.12)

v_j Specific rate of the jth relation (mmoles (g DW h)$^{-1}$)

$^*v^i$ Overall specific rate (or activity) of the ith reaction group (mmoles (g DW h)$^{-1}$)

v_{max} Maximum specific rate of an enzyme catalyzed reaction (mmoles h^{-1})

$\mathbf{v}$ Vector of reaction rates (or intracellular steady state fluxes) (mmoles (g DW h)$^{-1}$)

$\mathbf{v}_c$ Vector of non-measured reaction rates (mmoles (g DW h)$^{-1}$)

$\mathbf{v}_m$ Vector of measured reaction rates (mmoles (g DW h)$^{-1}$)

V Volume of the bioreactor (L)

x Biomass concentration (g L^{-1})

$X_{macro,i}$ Concentration of ith macromolecular pool (units) (g (g DW)$^{-1}$)

$X_{met,i}$ Concentration of ith intracellular metabolite

Y_{ij} Yield coefficient (mmoles j (mmole i)$^{-1}$)

Y_{ij}^{true} The true yield coefficient (mmoles j (mmole i)$^{-1}$)

Y_{xATP} ATP requirement for cell growth (mmoles ATP (g DW)$^{-1}$)

$Y_{xATP,growth}$ ATP requirement for cell synthesis (mmoles ATP (g DW)$^{-1}$)

$Y_{xATP,lysis}$ ATP requirement for cell growth dissipated due to cell lysis (mmoles ATP (g DW)$^{-1}$)

$Y_{xATP,leak}$ ATP requirement for leaks and futile cycles (mmoles ATP (g DW)$^{-1}$)

Z The phenomenological stoichiometry given by eq. (14.48)

GREEK LETTERS

α_{ji} Stoichiometric coefficient for the ith substrate in the jth reaction

$\mathbf{A}$ Matrix containing the stoichiometric coefficients for the substrates

β_{ji} Stoichiometric coefficient for the ith metabolic product in the jth reaction

$\mathbf{B}$ Matrix containing the stoichiometric coefficients for the metabolic products

χ Force ratio given by eq. (14.49)
χ_i Parameters in eq. (11.78)
δ Vector of measurement errors
ε Vector of residuals given by eq. (4.20)
$\varepsilon^i_{X_j}$ Elasticity coefficient given by eq. (11.11)
ϕ_i^j Metabolite amplification factor given by eq. (11.103)
Φ_i Dissipation function (kJ mole^{-1})
γ_{ji} Stoichiometric coefficient for the ith macromolecular pool in the jth reaction
Γ Matrix containing the stoichiometric coefficients for the macromolecular pools
η_{th} Thermodynamic efficiency
κ Generalized degree of reduction
μ Specific growth rate (h^{-1})
μ_i Chemical potential of the ith compound (kJ mole^{-1})
μ_i^0 Chemical potential of the ith compound at the reference state (kJ mole^{-1})
$\pi^i_{p_j}$ Parameter elasticity coefficient given by eq. (11.19)
τ Characteristic time (h)

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