

Contents

Preface	xv
Chapter 1. Introduction	1
Metabolic and Cellular Engineering in the Context of Bioprocess Engineering	4
Tools for Metabolic and Cellular Engineering	17
Engineering Cells for Specific Biotransformations	20
Current Trends: Biofuels and Biomass Interconversion Processes	23
Metabolic Areas that have been Subjected to MCE	27
Genome Sequencing, Comparative Genomics, and Biological Complexity	30
From DNA Sequence to Biological Function	32
Systems Biology	35
Systems Biology and the Complex Systems Approach	38
Networks in Systems Biology	40
Temporal and Spatial Scaling in Cellular Processes	44
Scaling in microbial and biochemical systems	47
Views of the Cell	49
Black and gray boxes: Levels of description of metabolic behavior in microorganisms	49
Transduction and Intracellular Signaling	55
Self-Organized Emergent Phenomena	57
Homeodynamics and Coherence	62
Functions of Rhythms and Clocks	66

Chapter 2. Matter and Energy Balances	69
Mass Balance	69
General formulation of mass balance	70
Integral and differential mass balances	72
Growth stoichiometry and product formation	72
Biomass and product yields	77
Electron balance	78
Theoretical oxygen demand	81
Opening the "Black Box": Mass balance as the basis of	
metabolic flux analysis	82
Anabolic fluxes	82
Catabolic fluxes	89
Energy Balance	93
Forms of energy and enthalpy	94
Calorimetric studies of energy metabolism	97
Heat of combustion	99
An energetic view of microbial metabolism	102
Chapter 3. Cell Growth and Metabolite Production:	
Basic Concepts	107
Microbial Growth under Steady and "Balanced"	
Conditions	107
Microbial Energetics under Steady-State Conditions	113
Growth Kinetics under Steady-State Conditions	115
The dilution rate	115
The dilution rate and biomass concentration	116
The Dilution Rate and the Growth-limiting Substrate	
Concentration	117
Biomass and Growth-limiting Substrate Concentration at Steady	
State	118
Metabolic Fluxes during Balanced and Steady-State	
Growth	121
Growth as a Balance of Fluxes	123
The Flux Coordination Hypothesis	126
Flux imbalance and cell cycle arrest	129

Redirecting Central Metabolic Pathways under Kinetic or Thermodynamic Control	131
Thermodynamic or Kinetic Control of Flux under Steady-State Conditions	132
Kinetic and Thermodynamic Limitations in Microbial Systems: Case Studies	134
<i>Saccharomyces cerevisiae</i>	134
Catabolite repression and cell cycle regulation in yeast . .	137
<i>Escherichia coli</i>	142
Metabolic Design of Cells as Catalysts	143
Increasing carbon flow to aromatic biosynthesis in <i>Escherichia coli</i>	143
Multipurpose engineered microbes	146
Chapter 4. Engineering of Process Performance	149
Biochemical Rationale of Growth and Product Formation . . .	149
A General Formalism for MFA	153
MFA Applications	154
MFA applied to prokaryotic organisms	155
MFA applied to lower eukaryotic organisms	158
Amphibolic pathways flux in different carbon sources . .	159
Interaction between carbon and nitrogen regulatory pathways in <i>S. cerevisiae</i>	160
MFA as applied to studying the performance of mammalian cells in culture	162
Bioenergetic and Physiological Studies in Batch and Continuous Cultures: Genetic or Epigenetic Redirection of Metabolic Fluxes	163
Introduction of heterologous metabolic pathways	163
Metabolic engineering of lactic acid bacteria for optimizing essential flavor compounds production	166
High throughput Bioenergetic and Physiological Studies of H ₂ Production by Algae	169
A metabolic switch elicited by the limitation of sulfur . . .	169
MFA under different growth conditions and high throughput metabolomics in plant cells	171

Flux analysis during H ₂ production using data from plant metabolomics	174
Metabolic engineering of microalgae	176
The engineered metabolome of <i>C. reinhardtii</i>	178
Rational Design of Microorganisms: Two Case Studies	179
Increase of Ethanol Production in Yeast	180
Phase I: Physiological, metabolic, and bioenergetic studies of different strains of <i>S. cerevisiae</i>	180
Phase II: Metabolic control analysis and metabolic flux analysis of the strain under the conditions defined in phase I	181
Yields and flux analysis	181
Metabolic control analysis	183
Phases III and IV: To obtain a recombinant yeast strain with an increased dose of PFK, and to assay the engineered strain in chemostat cultures under the conditions specified in phase I	186
Increase of L-threonine Production in <i>E. coli</i>	187
Appendix	189
Conditions for parameter optimization and simulation of a mathematical model of glycolysis	189
Chapter 5. Modeling Networks: Concepts and Tools	191
Cells as Networks of Processes	191
From “-Omics” to Functional Impact in Integrated Metabolic Networks	193
Networks Analyzed with Stoichiometric Models	195
Topological Analysis of Networks	202
Basic concepts on networks and graphs	205
Usefulness of the Analysis of Metabolic Networks in the Context of MCE	208
Kinetic Modeling in Microbial Physiology and Energetics	209
Modular building of kinetic models	209
Mathematical models behavior and tools: Reliability criteria	210
Model building process	211

Assembling the modules	214
Running simulations with the model: A combined experimental-computational approach	215
Metabolic Control Analysis of Networks	217
General concepts of MCA	217
MCA: Summation and connectivity theorems	222
Control and Regulation	224
Control of Metabolite Concentrations	226
Control of metabolites in relation to catabolite repression mutants of <i>S. cerevisiae</i>	227
MCA of Metabolic and Transport Networks	231
Analysis and Detection of Complex Nonlinear Behavior in Networks	233
Methods of Time Series Analysis	235
Relative Dispersional Analysis	235
Power Spectral Analysis	236
Applications to Time Series Analysis from Cardiomyocytes and Spontaneously Synchronized Continuous Yeast Cell Cultures	236
Cardiomyocytes	236
Yeast cultures	238
Complex Qualitative Behavior in Networks	239
Stability and Bifurcation Analyses	240
Emergent Spatiotemporal Behavior in Networks	243
Appendix	247
A simplified mathematical model to illustrate the Sauro–Westerhoff method of MCA	247
Chapter 6. Dynamic Aspects of Bioprocess Behavior	249
Transient and Oscillatory States of Continuous Culture	249
Mathematical model building	249
Transfer-function analysis and transient-response techniques	255
Theoretical transient response and approach to steady state	256
Substrate-inhibition model	257

Phase plane analysis	257
Transient Responses of Microbial Cultures to Perturbations	
of the Steady State	260
Dilution rate	260
Feed substrate concentration	260
Growth with two substrates	261
Temperature	261
Dissolved Oxygen	262
The meaning of steady-state performance in chemostat culture	262
Oscillatory Phenomena in Continuous Cultures	263
Oscillations as a consequence of equipment artifacts	263
Oscillations derived from feedback between cells and environmental parameters	264
Oscillations derived from intracellular feedback regulation	265
Glycolytic oscillations	265
Respiratory oscillations	266
Growth rate oscillations	267
Oscillations derived from interactions between different species in continuous culture	267
Oscillations due to synchronous growth and division	267
Self-synchronized continuous cultures of yeast	272
Chapter 7. Bioprocess Development with Plant Cells	281
MCE in Plants: Realities and Potentialities	282
Plant transformation for studies on metabolism and physiology	283
Emerging scenarios from -omics projects and systems biology	283
Improving Plants through Genetic Engineering	286
Improving plant resistance to chemicals, pathogens, and stress	287
Improving quality and quantity of plant products	289

Using genetic engineering to produce heterologous proteins in plants	294
Tools for the Manipulation and Transformation of Plants	295
Plant Metabolism: Matter and Energy Flows and the Prospect of MCA	299
Metabolic Compartmentation in Plant Cells	300
Carbon Assimilation, Partitioning, and Allocation	303
Carbon Fixation in Higher Plants	303
Carbon Partitioning within One Cell and between Source and Sink Tissues	310
Plants as a source of soluble and storage carbohydrates and sugar-alcohols	311
Plants as producers of lignocellulosic biomass	315
Plants as a source for oils	316
Distinctive characteristics of metabolic reactions in the cytoplasm of plant cells with relevance for carbon and energy partitioning	318
MCA and MFA Studies in Plants	324
Regulation and Control: Starch Synthesis: A Case Study	326
The Relevance of Unicellular Algae: Auto-, Hetero-, and Mixo-Trophy	330
Concluding Remarks	332
 Chapter 8. Animal Cell Culture Techniques	 335
General Remarks: Cells Cultured as Organisms	335
Mammalian Stem Cell Culture	337
Fish Cell Lines	337
Amphibian Cell Lines	338
Insect Cell Lines	338
Mammalian Cells	338
 Chapter 9. Metabolic and Cellular Engineering and Bioprocess Engineering in the Industrial Production of Therapeutic Proteins	 343

Bioprocess Engineering in the Industrial Production of Therapeutic Proteins	344
Bioprocess Design and Product Development	347
Stage 1: Molecule design	347
Stages 2 and 3: Host strain selection and process development	351
Stage 4: Risk assessment	352
Stage 5: Process characterization	355
 Chapter 10. Systems Biology of Metabolic and Cellular Engineering	 359
Biological Complexity and Systems Bioengineering	359
Cellular Systems Biology	360
An <i>In Silico</i> Cell: Multiple Temporal and Spatial Scales of Interacting Dynamic Systems in the Heart	361
Signaling Networks: Connecting and Modulating the Mass-Energy-Information Networks	366
Adaptation to Ischemic Conditions in the Heart	368
Components and mechanisms	368
Targets	369
Conditions for signaling activation	369
Physiological response	369
Adaptation to a glucose pulse in <i>S. cerevisiae</i>	370
Control and Regulation in Complex Networks	371
Systems Bioengineering	374
The Challenges Ahead	376
The Next Generation of Biofuels	377
Systems Bioengineering as Applied to Biomedicine	380
 Bibliography	 383
 Index	 425