Engineering Principles in Biotechnology

Wei-Shou Hu

Department of Chemical Engineering and Materials Science University of Minnesota, USA



Contents

Preface *xvii* About the Companion Website *xix*

1 An Overview of Bioprocess Technology and Biochemical

Engineering 1

- 1.1 A Brief History of Biotechnology and Biochemical Engineering 1
- 1.1.1 Classical Biotechnology 1
- 1.1.2 Recombinant DNA 4
- 1.1.3 A Typical Bioprocess 6
- 1.1.4 Biochemical Engineering and Bioprocess Technology 8
- 1.2 Industrial Organisms 10
- 1.2.1 Prokaryotes 12
- 1.2.1.1 Eubacteria and Archaea 12
- 1.2.2 Eukaryotic Microorganisms 12
- 1.2.2.1 Fungi 13
- 1.2.2.2 Algae 13
- 1.2.3 Multicellular Organisms and Their Cells 13
- 1.2.3.1 Insect Cells 13
- 1.2.3.2 Plant Cells, Tissues, and Organs 13
- 1.2.3.3 Animal Cells, Tissues, and Organs 14
- 1.2.4 Transgenic Plants and Animals 14
- 1.3 Biotechnological Products 15
- 1.3.1 Metabolic Process 15
- 1.3.2 Metabolites 18
- 1.3.3 Cells, Tissues, and Their Components 19
- 1.3.3.1 Viruses 20
- 1.3.4 Secreted Enzymes and Other Biopolymers 20
- 1.3.5 Recombinant DNA Products 20
- 1.3.5.1 Heterologous rDNA Proteins 20
- 1.3.6 Metabolic Engineering and Synthetic Pathways 22
- 1.4 Technology Life Cycle, and Genomics- and Stem Cell-Based New Biotechnology 23
- 1.4.1 The Story of Penicillin and the Life Cycle of Technology 23
- 1.4.2 Genomics, Stem Cells, and Transformative Technologies 25
 Further Reading 26
 Problems 26

viii	Contents

2 An Introduction to Industrial Microbiology and Cell

Biotechnology 29

- 2.1 Universal Features of Cells 29
- 2.2 Cell Membranes, Barriers, and Transporters 30
- 2.3 Energy Sources for Cells 31
- 2.3.1 Classification of Microorganisms According to Their Energy Source 32
- 2.4 Material and Informational Foundation of Living Systems 34
- 2.4.1 All Cells Use the Same Molecular Building Blocks 34
- 2.4.2 Genes 34
- 2.4.3 Genetic Information Processing 36
- 2.5 Cells of Industrial Importance 36
- 2.5.1 Prokaryotes 38
- 2.5.2 Eubacteria 38
- 2.5.2.1 Cell Wall and Cell Membrane 38
- 2.5.2.2 Membrane and Energy Transformation 40
- 2.5.2.3 Differentiation 41
- 2.5.3 Archaea 42
- 2.5.4 Eukaryotes 43
- 2.5.4.1 The Nucleus 44
- 2.5.4.2 Mitochondrion 45
- 2.5.4.3 Endoplasmic Reticulum and Golgi Apparatus 46
- 2.5.4.4 Other Organelles 47
- 2.5.4.5 Cytosol 48
- 2.6 Cells Derived from Multicellular Organisms 49
- 2.7 Concluding Remarks 50 Further Reading 50 Problems 50
- 3 Stoichiometry of Biochemical Reactions and Cell Growth 53
- 3.1 Stoichiometry of Biochemical Reactions 53
- 3.1.1 Metabolic Flux at Steady State 58
- 3.1.1.1 NAD/NADH Balance in Glycolysis 59
- 3.1.1.2 Oxidative Metabolism and NADH 60
- 3.1.2 Maximum Conversion of a Metabolic Product 63
- 3.2 Stoichiometry for Cell Growth 66
- 3.2.1 Cell Composition and Material Flow to Make Cell Mass 66
- 3.2.1.1 Composition and Chemical Formula of Cells 66
- 3.2.1.2 Material Flow for Biomass Formation 69
- 3.2.2 Stoichiometric Equation for Cell Growth 70
- 3.2.2.1 Yield Coefficient 71
- 3.3 Hypothetical Partition of a Substrate for Biomass and Product Formation 73
- 3.4 Metabolic Flux Analysis 74
- 3.4.1 Analysis of a Chemical Reaction System 74
- 3.4.1.1 Setting Up Material Balance Equations 74
- 3.4.1.2 Quasi-Steady State 76
- 3.4.1.3 Stoichiometric Matrix, Flux Vectors, and Solution 76
- 3.4.2 Analysis of Fluxes in a Bioreaction Network 77

- 3.4.3 Metabolic Flux Analysis on a Cellular System 81
- 3.4.3.1 Selecting Reactions for Analysis 81
- 3.4.3.2 Compartmentalization 83
- 3.4.3.3 Biomass 83
- 3.4.3.4 Limitations on Accounting of Materials 84
- 3.4.3.5 Solution and Analysis 84
- 3.5 Concluding Remarks 85 Further Reading 85 Nomenclature 86 Problems 86
- 4 Kinetics of Biochemical Reactions 95
- 4.1 Enzymes and Biochemical Reactions 95
- 4.2 Mechanics of Enzyme Reactions 96
- 4.3 Michaelis–Menten Kinetics 98
- 4.4 Determining the Value of Kinetic Parameters 101
- 4.5 Other Kinetic Expressions 104
- 4.6 Inhibition of Enzymatic Reactions 106
- 4.7 Biochemical Pathways 108
- 4.7.1 Kinetic Representation of a Reaction Pathway 108
- 4.7.2 Linearity of Fluxes in Biochemical Pathways 110
- 4.8 Reaction Network 114
- 4.9 Regulation of Reaction Rates 114
- 4.9.1 Flux Modulation by K_m 114
- 4.9.2 Allosteric Regulation of Enzyme Activities 115
- 4.9.3 Regulation at Transcriptional and Posttranslational Levels 117
- 4.9.4 Modulation of Resource Distribution through Reversible Reactions 118
- 4.10 Transport across Membrane and Transporters 120
- 4.10.1 Transport across the Cell Membrane 120
- 4.10.2 Transport of Electrolytes 121
- 4.10.3 Transport of Charged Molecules across Membrane 122
- 4.10.4 Types of Transporters 123
- 4.10.5 Kinetics of a Facilitated Transporter 124
- 4.11 Kinetics of Binding Reactions 126
- 4.11.1 Binding Reactions in Biological Systems 126
- 4.11.2 Dissociation Constant 127
- 4.11.3 Saturation Kinetics 128
- 4.11.4 Operator Binding and Transcriptional Regulation 129
- 4.11.5 Kinetics of Transcription and Translation 131
- 4.12 Concluding Remarks 135 Further Reading 136 Nomenclature 136 Problems 138
- 5 Kinetics of Cell Growth Processes 145
- 5.1 Cell Growth and Growth Kinetics 145
- 5.2 Population Distribution 148

```
x Contents
```

- 5.3 Description of Growth Rate 149
- 5.4 Growth Stage in a Culture 150
- 5.5 Quantitative Description of Growth Kinetics 151
- 5.5.1 Kinetic Description of Substrate Utilization 153
- 5.5.2 Using the Monod Model to Describe Growth in Culture 155
- 5.6 Optimal Growth 156
- 5.7 Product Formation 158
- 5.8 Anchorage-Dependent Vertebrate Cell Growth 159
- 5.9 Other Types of Growth Kinetics 161
- 5.10 Kinetic Characterization of Biochemical Processes 162
- 5.11 Applications of a Growth Model 163
- 5.12 The Physiological State of Cells 164
- 5.12.1 Multiscale Model Linking Biotic and Abiotic Phases 166
- 5.13 Kinetics of Cell Death 168
- 5.14 Cell Death and the Sterilization of Medium *169*
- 5.15 Concluding Remarks 171
 - Further Reading 172
 - Nomenclature 172 Problems 173
- 6 Kinetics of Continuous Culture 183
- 6.1 Introduction 183
- 6.2 Kinetic Description of a Continuous Culture 185
- 6.2.1 Balance Equations for Continuous Culture 185
- 6.2.2 Steady-State Behavior of a Continuous Culture 187
- 6.2.2.1 Monod Kinetics 187
- 6.2.2.2 Steady-State Concentration Profiles 187
- 6.2.2.3 Washout 189
- 6.2.3 Productivity in Continuous Culture 190
- 6.3 Continuous Culture with Cell Recycling 193
- 6.3.1 Increased Productivity with Cell Recycling 193
- 6.3.2 Applications of Continuous Culture with Cell Recycling 196
- 6.3.2.1 Low Substrate Levels in the Feed 196
- 6.3.2.2 Low Residual Substrate Concentration 197
- 6.3.2.3 Labile Product 197
- 6.3.2.4 Selective Enrichment of Cell Subpopulation 197
- 6.3.2.5 High-Intensity Mammalian Cell Culture 197
- 6.4 Specialty Continuous Cultures 199
- 6.4.1 Multiple-Stage Continuous Culture 199
- 6.4.2 Immobilized Cell Culture System 200
- 6.4.3 Continuous Culture with Mixed Populations 201
- 6.5 Transient Response of a Continuous Culture 202
- 6.5.1 Pulse Increase at the Substrate Level 203
- 6.5.2 Step Change in Feed Concentration 204
- 6.6 Concluding Remarks 205

Further Reading 205 Nomenclature 205 Problems 206

7 Bioreactor Kinetics 217

- 7.1 Bioreactors 217
- 7.2 Basic Types of Bioreactors 218
- 7.2.1 Flow Characteristics in Idealized Stirred-Tank (Well-Mixed) and Tubular (Plug Flow) Reactors 219
- 7.2.2 Reaction in an Idealized CSTR 220
- 7.2.3 Reaction in an Idealized PFR 222
- 7.2.4 Heterogeneous and Multiphasic Bioreactors Segregation of Holding Time 225
- 7.3 Comparison of CSTR and PFR 225
- 7.3.1 CSTR versus PFR in Conversion Yield and Reaction Rate 225
- 7.3.2 CSTR versus PFR in Terms of Nutrient Depletion and Scale-Up 226
- 7.3.3 CSTR versus PFR A Perspective from Residence Time Distribution 227
- 7.4 Operating Mode of Bioreactors 229
- 7.4.1 Batch Cultures 229
- 7.4.2 Fed-Batch Cultures 229
- 7.4.2.1 Intermittent Harvest 229
- 7.4.2.2 Fed-Batch 230
- 7.5 Configuration of Bioreactors 231
- 7.5.1 Simple Stirred-Tank Bioreactor 231
- 7.5.2 Airlift Bioreactor 233
- 7.5.3 Hollow-Fiber Bioreactor 233
- 7.6 Other Bioreactor Applications 233
- 7.7 Cellular Processes through the Prism of Bioreactor Analysis 235
- 7.8 Concluding Remarks 236 Further Reading 236
 - Nomenclature 237
 - Problems 237

8 Oxygen Transfer in Bioreactors 241

- 8.1 Introduction 241
- 8.2 Oxygen Supply to Biological Systems 242
- 8.3 Oxygen and Carbon Dioxide Concentration in Medium Henry's Law 243
- 8.4 Oxygen Transfer through the Gas–Liquid Interface 244
- 8.4.1 A Film Model for Transfer across the Interface 244
- 8.4.2 Concentration Driving Force for Interfacial Transfer 245
- 8.4.3 Mass Transfer Coefficient and Interfacial Area 246
- 8.5 Oxygen Transfer in Bioreactors 248
- 8.5.1 Material Balance on Oxygen in a Bioreactor 249
- 8.5.2 Oxygen Transfer in a Stirred Tank 251
- 8.6 Experimental Measurement of $K_L a$ and OUR 253

xii	Contents
-----	----------

- 8.6.1Determination of $K_L a$ in a Stirred-Tank Bioreactor2538.6.2Measurement of OUR and q_{02} 254
- 8.7 Oxygen Transfer in Cell Immobilization Reactors 256
- 8.8 Concluding Remarks 256 Further Reading 256 Nomenclature 256 Problems 258

9 Scale-Up of Bioreactors and Bioprocesses 265

- 9.1 Introduction 265
- 9.2 General Considerations in Scale Translation 266
- 9.2.1 Process and Equipment Parameters Affected by Scale-Up 266
- 9.2.2 Scale Translation for Product Development and Process Troubleshooting 266
- 9.2.3 How Scale-Up Affects Process Variables, Equipment, and Cellular Physiology 267
- 9.2.4 Scale-Up of Equipment and Geometrical Similarity 267
- 9.3 Mechanical Agitation 268
- 9.4 Power Consumption and Mixing Characteristics 269
- 9.4.1 Power Consumption of Agitated Bioreactors 269
- 9.4.2 Other Dimensionless Numbers 272
- 9.4.3 Correlation of Oxygen Transfer Coefficient 273
- 9.5 Effect of Scale on Physical Behavior of Bioreactors 273
- 9.6 Mixing Time 276
- 9.6.1 Nutrient Enrichment Zone: Mixing Time versus Starvation Time 276
- 9.6.2 Mixing Time 277
- 9.6.3 Mixing Time Distribution 278
- 9.7 Scaling Up and Oxygen Transfer 279
- 9.7.1 Material Balance on Oxygen in Bioreactor 279
- 9.7.1.1 Aeration Rate and the Oxygen Transfer Driving Force 280
- 9.8 Other Process Parameters and Cell Physiology 281
- 9.9 Concluding Remarks 282 Further Reading 283 Nomenclature 283 Problems 284

10 Cell Culture Bioprocesses and Biomanufacturing 289

- 10.1 Cells in Culture 289
- 10.2 Cell Culture Products 290
- 10.2.1 Vaccines 290
- 10.2.2 Therapeutic Proteins 291
- 10.2.3 Biosimilars 292
- 10.3 Cellular Properties Critical to Biologics Production 294
- 10.3.1 Protein Secretion 294
- 10.3.1.1 Folding in the Endoplasmic Reticulum 294
- 10.3.1.2 Membrane Vesicle Translocation and Golgi Apparatus 295
- 10.3.2 Glycosylation 296

- 10.3.3 Protein Secretion and Glycan Heterogeneity 296
- 10.4 Nutritional Requirements 299
- 10.4.1 Chemical Environment In Vivo and in Culture 299
- 10.4.2 Types of Media 300
- 10.4.2.1 Basal Medium and Supplements 300
- 10.4.2.2 Complex Medium, Defined Medium 301
- 10.5 Cell Line Development 301
- 10.5.1 Host Cells and Transfection 301
- 10.5.2 Amplification 302
- 10.6 Bioreactors 304
- 10.6.1 Roller Bottles 304
- 10.6.2 Stirred-Tank Bioreactors for Suspension Cells 305
- 10.6.3 Stirred-Tank Bioreactor with Microcarrier Cell Support 306
- 10.6.4 Disposable Systems 307
- 10.7 Cell Retention and Continuous Processes 307
- 10.7.1 Continuous Culture and Steady State 307
- 10.8 Cell Culture Manufacturing Productivity and Product Quality 308
- 10.8.1 Process and Product Quality 308
- 10.8.2 Product Life Cycle 309
- 10.8.3 Product Manufacturing 311
- 10.8.3.1 Platform Process 311
- 10.8.3.2 Manufacturing 311
- 10.9 Concluding Remarks 312 Further Reading 312 Problems 313

11 Introduction to Stem Cell Bioprocesses 319

- 11.1 Introduction to Stem Cells 319
- 11.2 Types of Stem Cells 320
- 11.2.1 Adult Stem Cells 320
- 11.2.1.1 Hematopoietic Stem Cells 321
- 11.2.1.2 Mesenchymal Stem Cells 323
- 11.2.1.3 Neuronal Stem Cells 323
- 11.2.2 Embryonic Stem Cells 324
- 11.2.3 Induced Pluripotent Stem Cells and Reprogramming 324
- 11.3 Differentiation of Stem Cells 326
- 11.4 Kinetic Description of Stem Cell Differentiation 328
- 11.5 Stem Cell Technology 331
- 11.6 Engineering in Cultivation of Stem Cells 332
- 11.7 Concluding Remarks 335 Further Reading 335 Nomenclature 336 Problems 336
- 12 Synthetic Biotechnology: From Metabolic Engineering to Synthetic Microbes 339
- 12.1 Introduction 339

- xiv Contents
 - 12.2 Generalized Pathways for Biochemical Production 340
 - 12.3 General Strategy for Engineering an Industrial, Biochemical-Producing Microorganism 342
 - 12.3.1 Genomics, Metabolomics, Deducing Pathway, and Unveiling Regulation 342
 - 12.3.2 Introducing Genetic Alterations 343
 - 12.3.3 Isolating Superior Producers 345
 - 12.3.3.1 Screening of Mutants with the Desired Phenotype 345
 - 12.3.3.2 Selection of Mutants with the Target Trait 345
 - 12.3.4 Mechanisms of Enhancing the Biosynthetic Machinery 347
 - 12.3.4.1 Relaxing the Constriction Points in the Pathway 347
 - 12.3.4.2 Channeling Precursor Supply 348
 - 12.3.4.3 Eliminating Product Diversion 350
 - 12.3.4.4 Enhancing Product Transport 350
 - 12.3.4.5 Rerouting Pathways 350
 - 12.3.5 Engineering Host Cells Beyond the Pathway 352
 - 12.3.5.1 Altering Substrate Utilization 352
 - 12.3.5.2 Manipulating the Time Dynamics of Production 352
 - 12.3.5.3 Increasing Product Tolerance 354
 - 12.4 Pathway Synthesis 356
 - 12.4.1 Host Cells: Native Hosts versus Archetypical Hosts 356
 - 12.4.2 Expressing Heterologous Enzymes to Produce a Nonnative Product 357
 - 12.4.3 Activating a Silent Pathway in a Native Host 359
 - 12.5 Stoichiometric and Kinetic Considerations in Pathway Engineering 359
 - 12.6 Synthetic Biology 367
 - 12.6.1 Synthetic (Cell-Free) Biochemical Reaction System 367
 - 12.6.2 Synthetic Circuits 369
 - 12.6.2.1 Artificial Genetic Circuits 369
 - 12.6.2.2 Synthetic Signaling Pathway 369
 - 12.6.3 Synthetic Organisms 371
 - 12.6.3.1 Minimum Genome and Reduced Genome 371
 - 12.6.3.2 Chemical Synthesis of a Genome 372
 - 12.6.3.3 Surrogate Cells from a Synthetic Genome 374
 - 12.7 Concluding Remarks 374 Further Reading 374 Problems 375

13 Process Engineering of Bioproduct Recovery 381

- 13.1 Introduction 381
- 13.2 Characteristics of Biochemical Products 382
- 13.3 General Strategy of Bioproduct Recovery 385
- 13.3.1 Properties Used in Bioseparation 385
- 13.3.2 Stages in Bioseparation 387
- 13.3.2.1 Cell and Solid Removal 387
- 13.3.2.2 Product Isolation (Capture) and Volume Reduction 387
- 13.3.2.3 Product Purification 388
- 13.3.2.4 Product Polishing 388

- 13.4 Unit Operations in Bioseparation 389
- 13.4.1 Filtration 389
- 13.4.2 Centrifugation 390
- 13.4.3 Liquid–Liquid Extraction 393
- 13.4.4 Liquid Chromatography 395
- 13.4.5 Membrane Filtration 396
- 13.4.6 Precipitation and Crystallization 397
- 13.5 Examples of Industrial Bioseparation Processes 398
- 13.5.1 Recombinant Antibody IgG 398
- 13.5.2 Penicillin 401
- 13.5.3 Monosodium Glutamate 404
- 13.5.4 Cohn Fractionation 404
- 13.6 Concluding Remarks 404 Further Reading 406 Nomenclature 407 Problems 408

14 Chromatographic Operations in Bioseparation 413

- 14.1 Introduction 413
- 14.2 Adsorbent 415
- 14.2.1 Types of Adsorbent 415
- 14.2.2 Ligand and Mechanism of Separation 418
- 14.2.3 Types of Liquid Chromatography 419
- 14.3 Adsorption Isotherm 420
- 14.3.1 Adsorption Equilibrium: Langmuir Isotherm 420
- 14.3.2 Isotherm Dynamics in Adsorption and Desorption 421
- 14.4 Adsorption Chromatography 425
- 14.4.1 Discrete-Stage Analysis 425
- 14.4.2 Breakthrough Curve 427
- 14.4.3 An Empirical Two-Parameter Description of a Breakthrough Curve 429
- 14.4.4 One-Porosity Model for an Adsorption Process 431
- 14.4.5 Elution of Solutes from an Adsorption Column 433
- 14.5 Elution Chromatography 435
- 14.5.1 Discrete-Stage Analysis 435
- 14.5.2 Determination of Stage Number 441
- 14.5.3 Effect of Stage Number and Number of Theoretical Plates 442
- 14.5.4 Two-Porosity Model, Mass Transfer Limitation 444
- 14.6 Scale-Up and Continuous Operation 447
- 14.6.1 Mass Transfer Limitation and the van Deemter Equation 447
- 14.6.2 Scale-Up of Chromatography 448
- 14.6.3 Continuous Adsorption and Continuous Elution Chromatography 450
- 14.7 Concluding Remarks 454
 Further Reading 454
 Nomenclature 454
 Problems 456

Index 471