

# Contents

<b>PREFACE</b>	<b>xvii</b>
----------------	-------------

<b>ABOUT THE AUTHORS</b>	<b>xxi</b>
--------------------------	------------

<b>Part 1 The Basics of Biology: An Engineer's Perspective</b>	<b>1</b>
--	----------

<b>1 WHAT IS A BIOPROCESS ENGINEER?</b>	<b>1</b>
---	----------

1.1. Biotechnology and Bioprocess Engineering	2
1.2. Differing Approaches to Research for Biologists and Engineers	3
1.3. The Story of Penicillin: How Biologists and Engineers Work Together	4
1.4. Bioprocesses: Regulatory Constraints	9
Suggestions for Further Reading	11
Questions	11

<b>2 AN OVERVIEW OF BIOLOGICAL BASICS</b>	<b>13</b>
---	-----------

2.1. Microbial Diversity	13
2.1.1. Naming Cells,	14
2.1.2. Viruses,	16
2.1.3. Prokaryotes,	18
2.1.4. Eukaryotes,	22



2.2.	Cell Construction	28
2.2.1.	<i>Amino Acids and Proteins</i> ,	29
2.2.2.	<i>Carbohydrates: Mono- and Polysaccharides</i> ,	37
2.2.3.	<i>Lipids, Fats, and Steroids</i> ,	42
2.2.4.	<i>Nucleic Acids, RNA, and DNA</i> ,	45
2.3.	Cell Nutrients	51
2.3.1.	<i>Macronutrients</i> ,	52
2.3.2.	<i>Micronutrients</i> ,	54
2.3.3.	<i>Growth Media</i> ,	55
2.4.	Summary	56
	Suggestions for Further Reading	58
	Questions	58

### 3 ENZYMES

61

3.1.	How Enzymes Work	62
3.2.	Enzyme Kinetics	63
3.2.1.	<i>Mechanistic Models for Simple Enzyme Kinetics</i> ,	65
3.2.2.	<i>Determining Rate Parameters for Michaelis-Menten Kinetics</i> ,	67
3.2.3.	<i>Models for More Complex Enzyme Kinetics</i> ,	71
3.2.4.	<i>Effects of pH and Temperature</i> ,	80
3.2.5.	<i>Insoluble Substrates</i> ,	83
3.2.6.	<i>Multiphase Enzymatic Reactions</i> ,	84
3.3.	Immobilized Enzyme Systems	86
3.3.1.	<i>Methods of Immobilization</i> ,	86
3.3.2.	<i>Diffusional Limitations in Immobilized Enzyme Systems</i> ,	91
3.3.3.	<i>Electrostatic and Steric Effects in Immobilized Enzyme Systems</i> ,	98
3.4.	Large-Scale Production of Enzymes	98
3.5.	Medical and Industrial Utilization of Enzymes	100
3.6.	Summary	103
	Suggestions for Further Reading	104
	Problems	104

### 4 HOW CELLS WORK

113

4.1.	The Central Dogma	114
4.2.	DNA Replication: Preserving and Propagating the Message	117
4.3.	Transcription: Sending the Message	119
4.4.	Translation: Going from Message to Product	123
4.4.1.	<i>Genetic Code: Universal Message</i> ,	123
4.4.2.	<i>Translation: How the Machinery Works</i> ,	124
4.4.3.	<i>Posttranslational Processing: Making the Product Useful</i> ,	125



4.5.	Metabolic Regulation	130
4.5.1.	<i>Genetic-Level Control: Which Proteins Are Synthesized?</i>	130
4.5.2.	<i>Metabolic Pathway Control</i>	133
4.6.	How the Cell Senses its Extracellular Environment	135
4.6.1.	<i>Transporting Small Molecules across Cellular Membranes</i>	135
4.6.2.	<i>Role of Cell Receptors in Metabolism and Cellular Differentiation</i>	137
4.7.	Summary	139
4.8.	Appendix: Example Regulation of Complex Pathways	140
	Suggestions for Further Reading	142
	Problems	143

## **5 MAJOR METABOLIC PATHWAYS**

**145**

5.1.	Bioenergetics	146
5.2.	Glucose Metabolism: Glycolysis and the TCA Cycle	149
5.3.	Respiration	152
5.4.	Control Sites in Aerobic Glucose Metabolism	154
5.5.	Metabolism of Nitrogenous Compounds	155
5.6.	Nitrogen Fixation	156
5.7.	Metabolism of Hydrocarbons	156
5.8.	Biodegradation of Xenobiotics	157
5.9.	Overview of Biosynthesis	158
5.10.	Overview of Anaerobic Metabolism	161
5.11.	Overview of Autotrophic Metabolism	163
5.12.	Summary	165
	Suggestions for Further Reading	166
	Questions	168

## **6 HOW CELLS GROW**

**169**

6.1.	Batch Growth	170
6.1.1.	<i>Quantifying Cell Concentration</i>	170
6.1.2.	<i>Growth Patterns and Kinetics in Batch Culture</i>	175
6.1.3.	<i>How Environmental Conditions Affect Growth Kinetics</i>	184
6.1.4.	<i>Heat Generation by Microbial Growth</i>	190
6.2.	Quantifying Growth Kinetics	191
6.2.1.	<i>Unstructured Nonsegregated Models</i>	192
6.2.2.	<i>Models for Transient Behavior</i>	201
6.2.3.	<i>Cybernetic Models</i>	207
6.3.	Cell Growth in Continuous Culture	208
6.3.1.	<i>Specific Devices for Continuous Culture</i>	208
6.3.2.	<i>The Ideal Chemostat</i>	210



6.3.3. *The Chemostat as a Tool*, 217

6.3.4. *Deviations from Ideality*, 218

6.4. Summary 219

Suggestions for Further Reading 219

Problems 220

## **7 STOICHIOMETRY OF MICROBIAL GROWTH AND PRODUCT FORMATION**

227

7.1. Coefficients for ATP Consumption and Oxygen 227

7.2. Stoichiometric Calculations 229

7.2.1. *Elemental Balances*, 230

7.2.2. *Degree of Reduction*, 230

7.3. Theoretical Predictions of Yield Coefficients 235

7.4. Estimation of Elemental Cell Composition 236

7.5. Stoichiometry by Oxidation-Reduction Half-Reactions 237

7.6. Thermodynamics of Biological Reactions 240

7.7. Summary 242

Suggestions for Further Reading 242

Problems 243

## **8 HOW CELLULAR INFORMATION IS ALTERED**

247

8.1. Evolving Desirable Biochemical Activities Through Mutation and Selection 247

8.1.1. *How Mutations Occur*, 248

8.1.2. *Selecting for Desirable Mutants*, 250

8.2. Natural Mechanisms for Gene Transfer and Rearrangement 252

8.2.1. *Genetic Recombination*, 252

8.2.2. *Transformation*, 254

8.2.3. *Transduction*, 254

8.2.4. *Episomes and Conjugation*, 256

8.2.5. *Transposons: Internal Gene Transfer*, 257

8.3. Genetically Engineering Cells 257

8.3.1. *Basic Elements of Genetic Engineering*, 258

8.3.2. *Genetic Engineering of Higher Organisms*, 265

8.3.3. *Genome Engineering*, 266

8.4. Genomics 267

8.4.1. *Experimental Techniques*, 268

8.4.2. *Computational Techniques*, 271

8.5. Summary 272

Suggestions for Further Reading 272

Problems 273



## 9 OPERATING CONSIDERATIONS FOR BIOREACTORS FOR SUSPENSION AND IMMOBILIZED CULTURES

275

- 9.1. Choosing the Cultivation Method 276
- 9.2. Modifying Batch and Continuous Reactors 278
  - 9.2.1. *Chemostat with Recycle*, 278
  - 9.2.2. *Multistage Chemostat Systems*, 281
  - 9.2.3. *Fed-Batch Operation*, 288
  - 9.2.4. *Perfusion Systems*, 293
  - 9.2.5. *Membrane Bioreactors*, 294
- 9.3. Immobilized Cell Systems 298
  - 9.3.1. *Active Immobilization of Cells*, 298
  - 9.3.2. *Passive Immobilization: Biological Films*, 303
  - 9.3.3. *Diffusional Limitations in Immobilized Cell Systems*, 304
  - 9.3.4. *Bioreactor Considerations in Immobilized Cell Systems*, 309
- 9.4. Hybrid Bioreactors: Attached and Suspended Cells 311
- 9.5. Solid-State Fermentations 313
- 9.6. Summary 316
- Suggestions for Further Reading 317
- Problems 318

## 10 SELECTION, SCALE-UP, OPERATION, AND CONTROL OF BIOREACTORS

323

- 10.1. Scale-Up and its Difficulties 323
  - 10.1.1. *Overview of Traditional Reactor Types*, 324
  - 10.1.2. *Reactors with Internal Mechanical Agitation*, 324
  - 10.1.3. *Bubble Column and Loop Reactor*, 329
  - 10.1.4. *Single-Use Bioreactors*, 330
  - 10.1.5. *Considerations in Aeration, Agitation, and Heat Transfer*, 331
  - 10.1.6. *Approaches to Scale-Up*, 337
  - 10.1.7. *Scale-Down and Microbioreactors*, 342
- 10.2. Bioreactor Instrumentation and Control 349
  - 10.2.1. *Instrumentation for Measurements of Active Fermentation*, 349
  - 10.2.2. *Using the Information Obtained*, 352
- 10.3. Sterilization of Process Fluids 356
  - 10.3.1. *The Kinetics of Death*, 356
  - 10.3.2. *Sterilization of Liquids*, 357
  - 10.3.3. *Sterilization of Gases*, 362



10.4.	Summary	364
	Suggestions for Further Reading	365
	Problems	366

## **11 RECOVERY AND PURIFICATION OF PRODUCTS**

**371**

11.1.	Strategies to Recover and Purify Products	371
11.2.	Separation of Insoluble Products	374
	11.2.1. Filtration,	374
	11.2.2. Centrifugation,	378
	11.2.3. Coagulation and Flocculation,	382
11.3.	Cell Disruption	382
	11.3.1. Mechanical Methods,	383
	11.3.2. Nonmechanical Methods,	384
11.4.	Separation of Soluble Products	385
	11.4.1. Liquid-Liquid Extraction,	385
	11.4.2. Aqueous Two-Phase Extraction,	389
	11.4.3. Precipitation,	390
	11.4.4. Dialysis,	392
	11.4.5. Reverse Osmosis,	393
	11.4.6. Ultrafiltration and Microfiltration,	395
	11.4.7. Cross-Flow Ultrafiltration and Microfiltration,	398
	11.4.8. Adsorption,	403
	11.4.9. Chromatography,	409
	11.4.10. Electrophoresis,	420
	11.4.11. Electrodialysis,	421
11.5.	Finishing Steps for Purification	422
	11.5.1. Crystallization,	422
	11.5.2. Drying,	423
11.6.	Integration of Reaction and Separation	424
11.7.	Summary	426
	Suggestions for Further Reading	426
	Problems	427

## **12 BIOPROCESS CONSIDERATIONS IN USING ANIMAL CELL CULTURES**

**431**

12.1.	Structure and Biochemistry of Animal Cells	431
12.2.	Methods Used for the Cultivation of Animal Cells	434
	12.2.1. Basic Techniques for Animal Cell Culture,	434
	12.2.2. Growth Media,	437
	12.2.3. Growth Dynamics for Animal Cells,	441
12.3.	Bioreactor Considerations for Animal Cell Culture	443
12.4.	Bioreactor Systems for Animal Cell Culture	444
	12.4.1. Nonstirred Reactor Systems,	444
	12.4.2. Systems for Entrapped Cells in Stirred Reactors,	445



12.4.3.	<i>Suspended Cultures</i> , 447	
12.5.	Products of Animal Cell Cultures	447
12.6.	Summary	448
	Suggestions for Further Reading	449
	Problems	450

### **13 BIOPROCESS CONSIDERATIONS IN USING PLANT CELL CULTURES**

**451**

13.1.	Why Plant Cell Cultures?	451
13.2.	Plant Cells in Culture Compared to Microbes	457
13.3.	Bioreactor Considerations	461
	13.3.1. <i>Bioreactors for Suspension Cultures</i> ,	462
	13.3.2. <i>Reactors Using Cell Immobilization</i> ,	463
	13.3.3. <i>Bioreactors for Organized Tissues</i> ,	466
13.4.	Economics of Plant Cell Tissue Cultures	467
13.5.	Summary	468
	Suggestions for Further Reading	468
	Problems	469

### **14 UTILIZING GENETICALLY ENGINEERED ORGANISMS**

**471**

14.1.	How the Product Influences Process Decisions	471
14.2.	Guidelines for Choosing Host-Vector Systems	474
	14.2.1. <i>Escherichia Coli</i> ,	475
	14.2.2. <i>Gram-Positive Bacteria</i> ,	477
	14.2.3. <i>Lower Eucaryotic Cells</i> ,	477
	14.2.4. <i>Mammalian Cells</i> ,	479
	14.2.5. <i>Insect Cell-Baculovirus System</i> ,	480
	14.2.6. <i>Transgenic Animals</i> ,	482
	14.2.7. <i>Transgenic Plants and Plant Cell Culture</i> ,	483
	14.2.8. <i>Cell-Free Protein Synthesis</i> ,	484
	14.2.9. <i>Comparison of Strategies</i> ,	484
14.3.	Process Constraints: Genetic Instability	485
	14.3.1. <i>Segregational Loss</i> ,	486
	14.3.2. <i>Plasmid Structural Instability</i> ,	488
	14.3.3. <i>Host Cell Mutations</i> ,	488
	14.3.4. <i>Growth-Rate-Dominated Instability</i> ,	489
14.4.	Avoiding Process Problems in Plasmid Design	490
14.5.	Predicting Host-Vector Interactions and Genetic Instability	493
14.6.	Regulatory Constraints on Genetic Processes	503
14.7.	Metabolic Engineering	506
14.8.	Synthetic and Systems Biology	509
14.9.	Protein Engineering	511
14.10.	Summary	513



## 15 MEDICAL APPLICATIONS OF BIOPROCESS ENGINEERING

519

### 15.1. Tissue Engineering 519

15.1.1. *What Is Tissue Engineering?*, 520

15.1.2. *Tissue-Engineered Skin Replacements*, 521

15.1.3. *Chondrocyte Culture for Cartilage Replacement*, 522

### 15.2. Gene Therapy Using Viral Vectors 523

15.2.1. *Models of Viral Infection*, 524

15.2.2. *Mass Production of Retrovirus*, 527

### 15.3. Bioreactors 528

15.3.1. *Stem Cells and Hematopoiesis*, 529

15.3.2. *Extracorporeal Artificial Liver*, 530

15.3.3. *Body-on-a-Chip Systems*, 530

### 15.4. Summary 531

Suggestions for Further Reading 532

Problems 532

## 16 BIOPROCESSES UTILIZING MIXED CULTURES

535

### 16.1. Major Classes of Interactions in Mixed Cultures 536

### 16.2. Simple Models Describing Mixed-Culture Interactions 539

### 16.3. Mixed Cultures in Nature 545

### 16.4. Industrial Utilization of Mixed Cultures 546

### 16.5. Biological Waste Treatment 549

16.5.1. *Biological Waste-Treatment Processes*, 551

16.5.2. *Advanced Wastewater Treatment Systems*, 566

16.5.3. *Conversion of Wastewater to Useful Products*, 571

### 16.6. Summary 572

Suggestions for Further Reading 572

Problems 573

## APPENDIX TRADITIONAL INDUSTRIAL BIOPROCESSES

577

### A.1. Anaerobic Bioprocesses 577

A.1.1. *Ethanol Production*, 578

A.1.2. *Lactic Acid Production*, 581

A.1.3. *Acetone-Butanol Production*, 584

### A.2. Aerobic Processes 586

A.2.1. *Citric Acid Production*, 586

A.2.2. *Production of Baker's Yeast*, 588

A.2.3. *Production of Penicillins*, 590

A.2.4. *Production of High-Fructose Corn Syrup*, 593



A.3.	Bioprocess Technologies: Biofuel and Bioenergy Production from Biomass	596
A.3.1.	<i>Production of Liquid Fuels</i>	596
A.3.2.	<i>Production of Gaseous Fuels from Biomass</i>	597
A.3.3.	<i>Bioelectricity Generation from Wastes Using Microbial Fuel Cells</i>	598
	Suggestions for Further Reading	600

## Preface

This third edition of *Bioprocess Engineering: Basic Concepts* updates the prior two editions. Although the principles of bioprocess engineering stated in the first two editions remain sound, the field has made considerable advances in improving the productivity, consistency, and safety of bioprocesses.

Some of the major changes in the book come from biology itself, with the development of tools that have greatly increased our ability to both understand and manipulate cell biology more effectively and cheaply. On the bioprocess side, tremendous advances have been made in production of biologicals with greater emphasis on processes incorporating animal cells. In particular, productivity in making many biopharmaceutical proteins has increased by several orders of magnitude over the last 15 years. The commercial use of animal cell culture has increased significantly over this period of time, and plant cell culture is starting to see commercial applications. Progress with microbes based on recombinant DNA and related technology have continued greatly. For any cell type, consistent upstream processing (culturing) of products remains challenging. The development of the concepts in systems and synthetic biology is now having an impact on the thinking of bioprocess engineers.

In this edition, we either introduce or expand discussion of the following issues:

- The role of small RNAs as regulators

- Cell-free processes