CONTENTS

1. Classical Biotechnology 1
1.0 Historical Perspective 1
1.1 Role of Krebs Cycle in Primary Metabolic Synthesis 4
1.2 Synthesis of Secondary Metabolites 14
1.3 Recombinant Cells 16
1.4 Scope 18
1.5 References 19

2. Enzyme Technology 21
2.0 General Considerations for Enzyme Systems 21
2.1 Historical Perspective 24
2.2 Coenzymes and Multienzymes 27
2.3 Kinetic Behavior of Immobilized Enzyme Systems 31
2.4 Kinetic Models for Immobilized Enzymes 32
2.5 References 46

3. Enzyme and Cell Based Reactors 52
3.0 Introduction 52
3.1 Carrier Matrix and Immobilization Method 52
3.2 Collagen Technology: First Generation 53
3.3 Enzyme Reactors 60
3.4 Lactose Processing Application 68
3.5 Biosensors 71
3.6 The Penetrant Time Lag in a Membrane Biosensor 73
3.7 Whole Cell Immobilization 75
3.8 Activity and Stability of Immobilized Cells 80
3.9 Immobilization of Isolated Organelles 82
3.10 Reactors with Whole Cells 83
3.11 Single Enzyme Type IMC Reactors 84
3.12 Effect of Mass Transfer on the Performance of Immobilized Cell Reactors 85
3.13 Fluidized Beds 87
3.14 Nomenclature 89
3.15 References 90

4. Bioprocess Kinetics 94
4.0 Introduction 94
4.1 Oxygen as the Limiting Substrate 96
4.2 Low Shear Environments 99
4.3 Membrane-Assisted Bioprocesses 102
4.4 General Framework 103
4.5 Immobilized Living Cell Systems 104
4.6 Analysis of Live Cell Reactors in Aerobic Processes 106
4.7 Idealized Reactor Performance Equations 107

4.8 Concept of a Dual Colony or Hybrid Reactor 111
4.9 Inducer/Substrate Transport Into the Immobilized Cells 113
4.10 General Mass Transfer Considerations 114
4.11 Anaerobic Processes 120
4.12 Carbon Flow Inside the Cell 121
4.13 Maintenance Energy 122
4.14 Schematic Description of the Model 123
4.15 Reactor Set Up 125
4.16 Model Equations 126
4.17 Hysteresis Due to Structure of the Beads 127
4.18 Enzyme Kinetics 129
4.19 Nomenclature 135
4.20 References 136

5. Bioprocess Optimization and Control: Antibiotic Case Study 140
5.0 Introduction to Economics of the Biotechnology Industry 140
5.1 Introduction to Process Optimization 142
5.2 Models for Secondary Metabolite Synthesis 144
5.3 Biosynthesis of Candicidin 146
5.4 References 156

6. The New Biotechnology 158
6.0 Recombinant Genes and Cloning 158
6.1 Operons 162
6.2 Lac Operon (E. Coli) 163
6.3 α-Amylase (B. Subtilis) 171
6.4 Yeasts 175
6.5 Transposons 177
6.6 Trp Operon 178
6.7 Applications of Genetic Engineering: Phenylalanine Case Study 181
6.8 References 185

7. Transcription and Translation of the Genetic Code 189
7.0 Transport Regulons 189
7.1 Permease Activity in E. coli 191
7.2 Recombinant Cell Culture 200
7.3 Bioreactor Studies 203
7.4 α-Amylase From Bacillus subtilis 206
7.5 Nomenclature 210
7.6 References 211

8. Immobilized Recombinant Microbial Cell Cultures and Bioreactors 214
8.0 Introduction 214
8.1 Immobilized Recombinant Cells 216

8.2 Production of β-Galactosidase 218
8.3 Strain Development 219
8.4 IMRC Bioreactors 220
8.5 IMRC Bioreactor Studies 222
8.6 Steady State IMRC Bioreactor Dynamics With Selection Pressure 224
8.7 Analysis 225
8.8 α-Amylase from B. subtilis 230
8.9 Coated Hollow Fibers 237
8.10 Nomenclature 241
8.11 References 241

9. Gene Expression With Animal Cells 244
9.0 Animal Cell Cultures 244
9.1 Cell Physiology 247
9.2 Mixing and Mechanical Stability for Anchorage Dependent and Independent Cells 250
9.3 Bioreactors for Recombinant Cells and Hybridoma Cells 253
9.4 Collagen Technology: Second Generation 258
9.5 References 262

10. Gene Expression With Plant Cells 265
10.0 Pathways 265
10.1 Plant Cell and Tissue Culture 286
10.2 Light Energy Coupling 301
10.3 Photosynthetic Electron Transport System 302
10.4 References 317

A. Appendix A 325
A.0 Bioreactor Transient Model: Lac Operon Expression 325
A.1 α-Amylase Studies 331
A.2 Nomenclature 343
A.3 References 344

B. Appendix B 346
B.0 Light Transport Model 346
B.1 Evaluation of Kinetic Parameters 363
B.2 References 374

Postscript 376

Author Index 377
Subject Index 385