

Contents

List of Contributors XXI

About the Series Editors XXXI

Preface XXXIII

Part I Enabling and Improving Large-Scale Bio-production 1

1	Industrial-Scale Fermentation	3
	<i>Hans-Peter Meyer, Wolfgang Minas, and Diego Schmidhalter</i>	
1.1	Introduction	3
1.2	Industrial-Scale Fermentation Today	5
1.2.1	Organisms Used in Large-Scale Fermentation	5
1.2.2	Contemporary Large-Scale Fermentation	7
1.2.3	Economic Aspects of Industrial Fermentation from a Market Perspective	14
1.2.4	The Drivers and the Future of Industrial Fermentation	15
1.3	Engineering and Design Aspects	18
1.3.1	Process Development – Scale-Up Starts at Laboratory Scale	18
1.3.2	Plant Design Aspects	19
1.3.2.1	General Aspects of Plant Design	19
1.3.2.2	Design Constraints and Guidelines	21
1.3.2.3	Seed Lines	24
1.3.2.4	Vessel Geometry	25
1.3.2.5	Mixing and Mass Transfer	27
1.3.2.6	Temperature Control and Heat Transfer	31
1.3.2.7	Oxygenation	32
1.4	Industrial Design Examples	36
1.4.1	Cephalosporin C Production	36
1.4.2	Monoclonal Antibody Production at the 10 m ³ Scale	39
1.4.3	Nonsterile Fermentations	42
1.5	Cost Analysis for the Manufacture of Biotechnological Products	42
1.5.1	Investment	42
1.5.2	Operational Cost, Cost of Manufacturing	43
1.5.3	Return on Invested Capital	47

1.6	Influence of Process- and Facility-Related Aspects on Cost Structure	47
1.6.1	Process-Related Aspects	48
1.6.2	Site-Related Aspects	48
	Acknowledgments	51
	References	52
2	Scale-Down: Simulating Large-Scale Cultures in the Laboratory	55
	<i>Alvaro R. Lara, Laura A. Palomares, and Octavio T. Ramírez</i>	
2.1	Introduction	55
2.2	Heterogeneities at Large Scale and the Need for Scaling Down	56
2.3	Bioreactor Scale-Down	58
2.4	Tools to Study Cell Responses to Environmental Heterogeneities	62
2.4.1	Scale-Down Simulators	62
2.4.1.1	One-Compartment Scale-Down Systems	63
2.4.1.2	Multicompartment Scale-Down Systems	64
2.4.2	Analytical Techniques	66
2.4.2.1	Metabolic Studies	66
2.4.2.2	Differential Gene Expression and Protein Accumulation	67
2.4.2.3	Physical Measurements	67
2.4.2.4	Mathematical Modeling	68
2.5	Physiological Effects of Environmental Heterogeneities	68
2.5.1	Negative Effects	68
2.5.1.1	Negative Effects on Animal Cells	70
2.5.2	Positive Effects	71
2.5.3	Further Observations	72
2.6	Improvements Based on Scale-Down Studies: Bioreactor Design and Cell Engineering	72
2.7	Perspectives	73
	Acknowledgment	74
	References	74
3	Bioreactor Modeling	81
	<i>Rob Mudde, Henk Noorman, and Matthias Reuss</i>	
3.1	Large-Scale Industrial Fermentations: Challenges for Bioreactor Modeling	81
3.1.1	Global Status	81
3.1.2	Perspectives	82
3.2	Bioreactors	83
3.2.1	Stirred-Tank Bioreactors	83
3.2.2	Bubble Columns and Air-Lift Reactors	86
3.2.3	Other Reactors	86
3.2.4	Bioreactor Modeling	87

3.3	Compartment and Hybrid Multizonal/Computational Fluid Dynamics Approaches for the Description of Large-Scale Bioreactor Phenomena	89
3.3.1	Compartment Models	89
3.3.2	Hybrid Multizonal/CFD Models	91
3.4	Computational Fluid Dynamics Modeling: Unstructured Continuum Approach (Euler–Euler)	92
3.4.1	Introduction	92
3.4.2	Single Phase	93
3.4.2.1	Turbulence Modeling	95
3.4.3	Two-Phase Flow	100
3.4.3.1	Approaches	100
3.4.3.2	Euler–Euler Model	100
3.4.3.3	Interaction Forces	102
3.4.3.4	Turbulence Modeling	103
3.4.4	CFD of Gassed Stirred Tanks	104
3.4.4.1	Bubble Size	105
3.4.4.2	Glucose Uptake	110
3.4.4.3	Oxygen Uptake – Distribution of Dissolved Oxygen	111
3.4.5	Summary of CFD	112
3.5	Computational Fluid Dynamics Modeling: Structured Segregated Approach (Euler–Lagrange)	114
3.5.1	Introduction	114
3.5.2	Euler–Lagrange Modeling	115
3.5.3	Metabolic Structuring	117
3.5.4	Model Simulations and Detailed Insight into Cell Responses to Dynamic Conditions in Large Bioreactors	118
3.6	Conclusion	122
3.7	Outlook	122
	References	124
4	Cell Culture Technology	129
	<i>Ralf Pörtner, Uwe Jandt, and An-Ping Zeng</i>	
4.1	Introduction	129
4.2	Overview of Applications for Cell Culture Products and Tissue Engineering	129
4.3	Fundamentals	131
4.3.1	Cell Sources	131
4.3.2	Cell Physiology and Kinetics for Process Engineering	132
4.3.3	Population Dynamics, Cell-Cycle Dependence, and Implications on Process Control	134
4.3.3.1	Separation Methods and Analytics	135
4.3.3.2	Population-Resolved Modeling and Data Treatment	136
4.3.3.3	Population-Resolved Online Monitoring and Process Control	138
4.3.4	Medium Design	139

4.4	Bioreactors for Cell Culture	140
4.4.1	Requirements	140
4.4.2	Bioreactors for Suspended Cells	142
4.4.3	Single-Use Bioreactors	144
4.4.4	Fixed-Bed and Fluidized-Bed Reactors	144
4.4.5	Hollow-Fiber and Membrane Reactors	145
4.4.6	Process Strategies and Control	145
4.5	Downstream	146
4.6	Regulatory and Safety Issues	150
4.7	Conclusions and Outlook	152
	References	152

Part II Getting Out More: Strategies for Enhanced Bioprocessing 159

5 Production of Fuels and Chemicals from Biomass by Integrated Bioprocesses 161

Tomohisa Hasunuma and Akihiko Kondo

5.1	Introduction	161
5.2	Utilization of Starchy Biomass	163
5.2.1	Pretreatment and Enzymatic Hydrolysis of Starch	163
5.2.2	Consolidated Bioprocessing for Starch Utilization	164
5.3	Utilization of Lignocellulosic Biomass	166
5.3.1	Pretreatment and Enzymatic Hydrolysis of Lignocellulose	166
5.3.2	Consolidated Bioprocessing for Lignocellulose Utilization	167
5.3.2.1	Introduction	167
5.3.2.2	Production of Chemicals with Native Cellulase-Producing Microbes	168
5.3.2.3	Production of Chemicals with Recombinant Cellulose-Utilizing Microbes	169
5.4	Conclusions and Perspectives	177
	Acknowledgment	177
	References	178

6 Solid-State Fermentation 187

Reeta Rani Singhania, Anil Kumar Patel, Leya Thomas, and Ashok Pandey

6.1	Introduction	187
6.2	Fundamentals Aspects of SSF	188
6.2.1	Selection of Microorganisms	188
6.2.2	Specific Growth Rate	189
6.2.2.1	Biomass Measurement	192
6.3	Factors Affecting Solid-State Fermentation	193
6.3.1	Moisture	193
6.3.2	Water Activity	193
6.3.3	Temperature	194

6.3.4	pH	194
6.3.5	Inoculum Type	194
6.3.6	Substrates	194
6.3.6.1	Particle Size	195
6.3.7	Aeration and Agitation	196
6.4	Scale-Up	196
6.4.1	Large-Scale Inoculum Development	196
6.4.2	Medium Sterilization	196
6.4.3	Aeration and Agitation	197
6.4.4	Heat Removal and Moisture Balance	197
6.4.5	pH Control	198
6.5	Product Recovery	198
6.6	Bioreactor Designing	198
6.6.1	Shallow-Tray Fermenter	199
6.6.2	Column/Fixed-Bed Fermenters	199
6.6.3	Rotating-Drum Bioreactors	199
6.7	Kinetics and Modeling	200
6.8	Applications	201
6.9	Challenges in SSF	202
6.10	Summary	203
	References	203
7	Cell Immobilization: Fundamentals, Technologies, and Applications	205
	<i>Xumeng Ge, Liangcheng Yang, and Jianfeng Xu</i>	
7.1	Introduction	205
7.2	Fundamentals of Cell Immobilization	206
7.3	Immobilization with Support Materials	207
7.3.1	Surface Attachment	208
7.3.1.1	Adsorption	208
7.3.1.2	Covalent Binding	209
7.3.1.3	Biofilm Formation	209
7.3.2	Entrapment	210
7.3.2.1	Entrapment in Gel Matrixes	210
7.3.2.2	Entrapment in Porous Particles	210
7.3.3	Encapsulation	211
7.3.4	Membrane Retention	212
7.4	Self-Immobilization	212
7.4.1	Microorganisms	213
7.4.1.1	Prokaryotic Cells	213
7.4.1.2	Eukaryotic Cells	214
7.4.2	Plant Cells	218
7.5	Immobilized Cells and their Applications	218
7.5.1	Microorganisms	219
7.5.2	Plant Cells	221

7.5.3	Mammalian and Insect cells	221
7.6	Bioreactors for Cell Immobilization	225
7.6.1	Stirred-Tank Bioreactor	226
7.6.2	Packed-Bed Bioreactor	227
7.6.3	Fluidized-Bed Bioreactor	227
7.6.4	Air-Lift Bioreactor	228
7.6.5	Membrane Bioreactor	228
7.7	Challenges and Recommendations for Future Research	229
7.8	Conclusions	230
	References	231

Part III Molecules for Human Use: High-Value Drugs, Flavors, and Nutraceuticals 237

8	Anticancer Drugs	239
	<i>Le Zhao, Zengyi Shao, and Jacqueline V Shanks</i>	
8.1	Natural Products as Anticancer Drugs	239
8.2	Anticancer Drug Production	239
8.2.1	Production Systems	239
8.2.2	Approaches for Improving Production	241
8.2.3	Gene Discovery	242
8.3	Important Anticancer Natural Products	243
8.3.1	Vinca Alkaloids	243
8.3.2	Taxane Diterpenoids	250
8.3.3	Podophyllotoxin Lignans	256
8.3.4	Camptothecin Quinoline Alkaloids	258
8.4	Prospects	261
8.4.1	Identification of Intermediates in the Biosynthetic Pathways of Anticancer Drugs	261
8.4.2	Discovery of Unknown Genes in Biosynthetic Pathways	262
8.4.3	Production of Anticancer Drugs in Microbial Hosts	262
	References	263
9	Biotechnological Production of Flavors	271
	<i>Maria Elisabetta Brenna and Fabio Parmeggiani</i>	
9.1	History	271
9.2	Survey on Today's Industry	272
9.3	Regulations	273
9.4	Flavor Production	274
9.5	Biotechnological Production of Flavors	275
9.5.1	Traditional Fermentations	275
9.5.2	<i>De novo</i> Synthesis	276
9.5.3	Bioconversions	277
9.6	Vanillin	277
9.6.1	From Eugenol	278

9.6.2	From Isoeugenol	278
9.6.3	From Ferulic Acid	280
9.6.4	From Lignin	281
9.7	2-Phenylethanol	281
9.8	Benzaldehyde	283
9.9	Lactones	285
9.10	Raspberry Ketone	289
9.11	Green Notes	291
9.12	Nootkatone	293
9.13	Future Perspectives	296
	References	297
10	Nutraceuticals (Vitamin C, Carotenoids, Resveratrol)	309
	<i>Sanjay Guleria, Jingwen Zhou, and Mattheos A.G. Koffas</i>	
10.1	Introduction	309
10.2	Vitamin C	310
10.2.1	Production of L-AA by Chemical Synthesis	311
10.2.2	Production of L-AA by a Two-Step Fermentation Process	311
10.2.3	Classical Two-Step Fermentation Process	312
10.2.4	New Two-Step Fermentation Process	313
10.2.5	Production of L-AA by a One-Step Fermentation Process	314
10.2.6	Classical Two-Step Fermentation Process-Based Attempts	314
10.2.7	New Two-Step Fermentation Process-Based Attempts	316
10.2.8	Reconstruction of L-AA Biosynthesis Pathway from Higher Organisms in Microorganisms	316
10.3	Carotenoids	317
10.3.1	Biosynthesis of Carotenoids	319
10.3.2	Metabolic Engineering of Carotenoid Biosynthesis in Microbes	321
10.4	Resveratrol	323
10.4.1	Biosynthesis of Resveratrol and Its Derivatives	324
10.4.2	Metabolic Engineering of Resveratrol and its Derivatives	327
10.5	Future Perspectives	329
	References	330
	Part IV Industrial Amino Acids	337
11	Glutamic Acid Fermentation: Discovery of Glutamic Acid-Producing Microorganisms, Analysis of the Production Mechanism, Metabolic Engineering, and Industrial Production Process	339
	<i>Takashi Hirasawa and Hiroshi Shimizu</i>	
11.1	Introduction	339
11.2	Discovery of the Glutamic Acid-Producing Bacterium <i>C. glutamicum</i>	340
11.2.1	Glutamic Acid Production Prior to the Discovery of Glutamic Acid-Producing Microorganisms	340

- 11.2.2 Discovery of *C. glutamicum*, a Glutamic Acid-Producing Bacterium 340
 - 11.2.3 Characteristics of *C. glutamicum* 342
 - 11.3 Analysis of the Mechanism of Glutamic Acid Production by *C. glutamicum* 342
 - 11.3.1 Relationship between Cell-Surface Structure and Glutamic Acid Production in *C. glutamicum* 343
 - 11.3.2 Metabolic Regulation during Glutamic Acid Overproduction in *C. glutamicum* 345
 - 11.3.2.1 Biosynthesis of Glutamic Acid in *C. glutamicum* 345
 - 11.3.2.2 Relationship between Enzyme Activity of the 2-Oxoglutarate Dehydrogenase Complex and Glutamic Acid Production 346
 - 11.3.2.3 OdhI Decreases the Enzymatic Activity of the 2-Oxoglutarate Dehydrogenase Complex 347
 - 11.3.2.4 Anaplerotic Reactions in Glutamic Acid Overproduction 348
 - 11.3.3 Involvement of a Mechanosensitive Channel, NCgl1221, in Glutamic Acid Secretion in *C. glutamicum* 349
 - 11.4 Metabolic Engineering of *C. glutamicum* for Glutamic Acid Production 350
 - 11.4.1 Metabolic Engineering 350
 - 11.4.2 Metabolic Flux Analysis in Glutamic Acid Production 350
 - 11.4.2.1 Analysis of the Impact of Activities of Enzymes Related to Glutamic Acid Production on the Flux of Glutamic Acid Production 351
 - 11.4.2.2 Use of ¹³C-MFA to Investigate the Importance of Anaplerotic Reactions to Glutamic Acid Production 351
 - 11.4.3 Metabolic Engineering for Improvement of Glutamic Acid Production 351
 - 11.5 Glutamic Acid Fermentation by Other Microorganisms 352
 - 11.6 Industrial Process of Glutamic Acid Production 353
 - 11.7 Future Perspectives 354
 - References 355
-
- 12 **L-Lysine** 361
 - Volker F. Wendisch*
 - 12.1 Uses of L-Lysine 361
 - 12.1.1 Feed Use of Amino Acids 361
 - 12.1.2 Economic Importance and Means of Production of L-Lysine 362
 - 12.2 Biosynthesis and Production of L-Lysine 363
 - 12.2.1 L-Lysine Biosynthesis 363
 - 12.2.2 Strain Development for the Production of L-Lysine 363
 - 12.2.2.1 L-Lysine Transport 365
 - 12.2.2.2 De-bottlenecking L-Lysine Biosynthesis 366
 - 12.2.2.3 NADPH Supply for L-Lysine Production 366
 - 12.2.2.4 Reduction of Byproducts of L-Lysine Production 367
 - 12.2.2.5 Precursor Supply for L-Lysine Production 367

- 12.2.3 Industrial Processes of L-Lysine Production 368
- 12.2.4 Flexible Feedstock Concept of *C. glutamicum*: Engineering Carbon Source Utilization 369
 - 12.2.4.1 Molasses, Glucose, Fructose, Sucrose, and Starch 370
 - 12.2.4.2 Lignocellulosics, Cellulose, Xylose, Arabinose, Acetate, Galactose 371
 - 12.2.4.3 Silage Juice and Lactic Acid 373
 - 12.2.4.4 Amino Sugars 373
 - 12.2.4.5 Dicarboxylic Acids 374
 - 12.2.4.6 Glycerol 374
- 12.3 The Chassis Concept: Biotin Prototrophy and Genome Reduction 374
 - 12.3.1 Engineering Biotin Prototrophic *C. glutamicum* 375
 - 12.3.2 Genome-Streamlined *C. glutamicum* Strains 375
- 12.4 L-Lysine Biosensors for Strain Selection and on-Demand Flux Control 377
 - 12.4.1 Transcriptional Regulators as Diagnostic Metabolite Sensors for Screening 377
 - 12.4.2 Riboswitches as Metabolite Sensors for on-Demand Metabolic Flux Control 379
- 12.5 Perspective 380
- References 380

Part V Bio-Based Monomers and Polymers 391

- 13 **Diamines for Bio-Based Materials** 393
Judith Becker and Christoph Wittmann
 - 13.1 Introduction 393
 - 13.2 Diamine Metabolism in Bacteria 395
 - 13.3 Putrescine – 1,4-Diaminobutane 395
 - 13.3.1 Metabolism of Putrescine 396
 - 13.3.2 Biosynthesis and Pathway Regulation 396
 - 13.3.3 Metabolic Engineering for Putrescine Production 398
 - 13.4 Cadaverine – 1,5-Diaminopentane 399
 - 13.4.1 Metabolism of Diaminopentane 399
 - 13.4.2 Biosynthesis and Pathway Regulation 400
 - 13.4.3 Metabolic Engineering for Cadaverine Production 400
 - 13.4.4 Bio-Based Polyamide PA5.10 – A Success Story 403
 - 13.5 Conclusions and Perspectives 403
 - References 404
- 14 **Microbial Production of 3-Hydroxypropionic Acid** 411
Yokimiko David, Young Hoon Oh, Mary Grace Baylon, Kei-Anne Baritugo, Jeong Chan Joo, Cheol Gi Chae, You Jin Kim, and Si Jae Park
 - 14.1 Introduction 411

14.2	3-HP Obtained from Native Producers	413
14.2.1	3-HP as an Intermediate of CO ₂ Fixation	413
14.2.2	Degradation Pathways	415
14.2.2.1	Acrylic Acid	415
14.2.2.2	Pyrimidines (Uracil and Thymine)	415
14.2.3	3-HP as a Nematicide	417
14.3	Synthesis of 3-HP from Glucose	417
14.4	Synthesis of 3-HP from Glycerol	421
14.4.1	CoA-Independent <i>dha</i> Operon	422
14.4.2	CoA-Dependent <i>pdu</i> Operon	425
14.4.3	Redirecting the Flux toward 3-HP Production	426
14.4.4	<i>K. pneumoniae</i> as a Host for Glycerol-Derived 3-HP Production	426
14.4.5	3-HP Production from Glycerol in Recombinant <i>E. coli</i>	431
14.5	Bridging the Gap Between Glucose and Glycerol in 3-HP Production	437
14.6	Other Strains for 3-HP Production from Glycerol	438
14.7	Limitations of 3-HP Synthesis	440
14.8	Conclusions and Future Prospects	442
	Acknowledgments	443
	References	444
15	Itaconic Acid – An Emerging Building Block	453
	<i>Matthias G. Steiger, Nick Wierckx, Lars M. Blank, Diethard Mattanovich, and Michael Sauer</i>	
15.1	Background, History, and Economy	453
15.2	Biosynthesis of Itaconic Acid	455
15.2.1	<i>Aspergillus terreus</i>	455
15.2.2	Genes and Enzymes Involved in the Biosynthesis of Itaconic Acid in <i>A. terreus</i>	455
15.2.3	Genes and Enzymes Involved in the Biosynthesis of Itaconic Acid in <i>Ustilago maydis</i>	459
15.3	Production Conditions for Itaconic Acid	459
15.4	Physiological Effects and Metabolism of Itaconic acid	461
15.5	Metabolic Engineering for Itaconic Acid Production	462
15.6	Outlook	467
	Acknowledgments	468
	References	469
	Part VI Top-Value Platform Chemicals	473
16	Microbial Production of Isoprene: Opportunities and Challenges	475
	<i>Huibin Zou, Hui Liu, Elhussiny Aboulnaga, Huizhou Liu, Tao Cheng, and Mo Xian</i>	
16.1	Introduction	475

16.2	The Milestones of Isoprene Production	476
16.3	Microbial Production of Isoprene: Out of the Laboratory	477
16.3.1	Advantages of Bioisoprene Against Petroleum-Derived Isoprene	477
16.3.2	Metabolic Pathways and Key Enzyme of Bioisoprene	477
16.3.3	Metabolic Engineering of MVA and MEP Pathways for Microbial Production of Isoprene	480
16.3.4	Substrate for the Microbial Production of Isoprene	481
16.3.5	Evaluation of Isoprene Biosynthetic Process from Different Substrates	482
16.3.6	Chassis Strains for the Microbial Production of Isoprene	485
16.3.7	Recovery Techniques for the Gas-Phase Bioisoprene	486
16.3.8	Scale-up Fermentation and Process Control of Bioisoprene	487
16.4	Main Challenges for Bioisoprene Production	489
16.5	Future Prospects	491
16.5.1	Rational Design of Central Metabolic Pathway to Increase the Yield and Productivity of Isoprene	491
16.5.2	Improving the Yield via Metabolic Pathways (MVA/MEP) Engineering	492
16.5.3	Improving the Intermediate Precursors via Enzyme Engineering	494
16.5.4	Novel Substrates for Bioisoprene	494
16.5.5	Integration of Bio and Chemo Substrates and Process for Isoprene Production	495
16.5.6	Novel Hosts for Isoprene Production	495
16.5.7	Exploring Anaerobic Routes	496
16.5.8	Biosynthesis of Value-Added Isoprene Derivatives	497
	Acknowledgments	498
	References	498
17	Succinic Acid	505
	<i>Jung Ho Ahn, Yu-Sin Jang, and Sang Yup Lee</i>	
17.1	Introduction	505
17.2	Development of Succinic Acid Producers and Fermentation Strategies	506
17.2.1	<i>Actinobacillus succinogenes</i>	507
17.2.2	<i>Anaerobiospirillum succiniciproducens</i>	510
17.2.3	<i>Corynebacterium glutamicum</i>	512
17.2.4	<i>Escherichia coli</i>	515
17.2.5	<i>Mannheimia succiniciproducens</i>	526
17.2.6	<i>Saccharomyces cerevisiae</i>	530
17.3	Succinic Acid Recovery and Purification	533
17.3.1	Precipitation	533
17.3.2	Electrodialysis	534

- 17.3.3 Reactive Extraction 535
- 17.3.4 Adsorption 536
- 17.4 Summary 536
- Acknowledgments 537
- References 537

Part VII Biorenewable Fuels 545

- 18 Ethanol: A Model Biorenewable Fuel 547**
 - Tao Jin, Jieni Lian, and Laura R. Jarboe*
 - 18.1 Introduction 547
 - 18.2 Metabolic Engineering: Design, Build, Test, Learn 549
 - 18.2.1 Design: Metabolic Pathway Engineering 550
 - 18.2.1.1 Introduction of a Foreign Pathway to Enable Non-native Substrate Utilization 550
 - 18.2.1.2 Introduction of a Foreign Pathway to Enable Homoethanol Production 552
 - 18.2.1.3 Selection of Metabolic Pathways for Modification 554
 - 18.2.1.4 Metabolic Engineering to Enable Mixed-Substrate Utilization 554
 - 18.2.1.5 Selection of Pathway Components for Tuning 555
 - 18.2.2 Design: Membrane Engineering for Improved Tolerance 555
 - 18.2.3 Build: Targeted Genetic Manipulation Techniques 556
 - 18.2.3.1 One-Step Chromosomal Editing of *E. coli* 556
 - 18.2.3.2 Shuttle Vectors for *S. cerevisiae* Engineering 556
 - 18.2.3.3 CRISPR/Cas 9 557
 - 18.2.4 Build: Evolutionary Strain Improvement 557
 - 18.2.4.1 Genome-Wide Evolution for Improved Tolerance and Production 557
 - 18.2.4.2 Enzyme Evolution to Enable Nonrecombinant Homoethanol Production 558
 - 18.2.5 Test: Screening of Expression Libraries 559
 - 18.2.5.1 Expression Libraries Containing Sequence Variants of a Preselected Gene 559
 - 18.2.5.2 Expression Libraries that Alter Gene Abundance 560
 - 18.2.5.3 Expression Libraries that Vary Genomic Integration Site 560
 - 18.2.6 Learn: Identifying Strategies and Targets for the Next Design Stage 561
 - 18.2.6.1 Reverse Engineering of Improved Strains 561
 - 18.2.6.2 Learn: Identification of Metabolic Burdens During Production 562
 - 18.3 Biomass Deconstruction 563
 - 18.4 Closing Remarks 564
 - Acknowledgments 564
 - References 564

19	Microbial Production of Butanols	573
	<i>Sio Si Wong, Luo Mi, and James C. Liao</i>	
19.1	Introduction	573
19.2	A Historical Perspective of <i>n</i> -Butanol Production	574
19.3	ABE Fermentation	575
19.3.1	The Biochemistry of ABE Fermentation	575
19.3.2	Developing Genetics Tools in <i>Clostridium acetobutylicum</i>	577
19.3.3	Metabolic Engineering of <i>Clostridium acetobutylicum</i> for Butanol Fermentation	578
19.4	<i>n</i> -Butanol Production in Non-native Producers	580
19.4.1	Rationale for Using Non-native Producers	580
19.4.2	Pathways for <i>n</i> -Butanol Biosynthesis	580
19.4.3	Improved <i>n</i> -Butanol Production with Driving Forces	582
19.5	Isobutanol Production	583
19.5.1	The Biochemistry of Isobutanol Production	583
19.5.2	Isobutanol Production from Sugar	584
19.5.3	Isobutanol Production from Cellulose	586
19.5.4	Isobutanol Production from CO ₂	586
19.5.5	Isobutanol Production from Waste Protein	587
19.5.6	Isobutanol Tolerance of <i>E. coli</i>	588
19.5.7	Other Products from the Keto-Acid Pathway	588
19.6	Summary and Outlook	589
	Acknowledgments	589
	References	589
	Index	597