

Contents

Preface XV

List of Contributors XVII

The Scope and Impact of Industrial Biotechnology 1

Wim Soetaert and Erick J. Vandamme

1	History of Industrial Biotechnology	17
	<i>Arnold L. Demain</i>	
1.1	Early History	17
1.2	The Penicillin Story	22
1.3	The Coming of the Cephalosporins	29
1.4	The Waksman Era	31
1.5	Strain Improvement	33
1.6	Semi-Synthetic Antibiotics to Combat Resistant Microbes	35
1.7	The Primary Metabolites	36
1.7.1	Amino Acids	36
1.7.2	Nucleotides	39
1.7.3	Vitamins	39
1.7.4	Organic Acids	41
1.7.5	Alcohols	43
1.7.6	Polymers	45
1.7.7	Specialty Sugars, Sugar Alcohols, L-Sugars, Oligosugars, Novel Extracellular Polysaccharides, Biopigments, Cosmetics Including Fragrants, and Microbial Enzymes for Chiral Synthesis and Other Applications	46
1.8	The Shift from Antibiotics to Pharmacological Agents	46
1.8.1	Enzyme Inhibitors	47
1.8.2	Immunosuppressants	49
1.8.3	Antitumor Agents	51
1.8.4	Ergot Alkaloids	51
1.8.5	Agricultural Compounds	51
1.9	The Biopharmaceutical Revolution	53

1.9.1	Human Insulin (Novolin, Humulin)	57
1.9.2	Erythropoietin (Epogen, Procrit)	58
1.9.3	Interferons	58
1.9.4	Human Growth Hormone (Somatotropin, Somatropin; Humatrope, Nutropin, Protropin, Somatren, Serostim)	58
1.9.5	Tissue Plasminogen Activator (Activase, Alteplase)	58
1.9.6	Interleukins	59
1.9.7	Factor VIII	59
1.9.8	Colony-Stimulating Factors	59
1.9.9	Human DNase (Pulmozyme)	59
1.9.10	Glucocerebrosidase (Cerezyme)	59
1.9.11	Monoclonal Antibodies	60
1.9.12	Additional Biopharmaceuticals	60
1.10	Recombinant Hosts	61
1.10.1	<i>E. coli</i>	61
1.10.2	Yeasts	62
1.10.3	Molds	62
1.10.4	Insect Cells	63
1.10.5	Mammalian Cells	63
1.10.6	Transgenic Animals	63
1.10.7	Transgenic Plants	64
1.11	Enzymes	64
1.12	Bioconversions	67
1.13	Vaccines	67
1.14	Systems Microbiology	67
	References	68

2 Industrial Systems Biology 79

José Manuel Otero and Jens Nielsen

2.1	Introduction	79
2.2	Industrial Biotechnology	80
2.3	Market Drivers for Industrial Biotechnology	85
2.4	Industrial Systems Biology	86
2.5	Metabolic Models	89
2.5.1	Microbial Metabolism—A Historical Perspective	89
2.5.2	Genome Sequencing and Functional Genomics	93
2.6	Reconstructed Metabolic Network Models	105
2.6.1	Introduction	105
2.6.2	Genome-Scale Reconstructed Network Process	115
2.7	Industrial Systems Biology Case Studies	121
2.7.1	A Mature and Developed Industrial Biotechnology Product: Bioethanol	121
2.7.2	A Recently Launched and Rapidly Growing Industrial Biotechnology Product: 1,3-Propanediol	125
2.7.3	An In-Development Industrial Biotechnology Product: Succinic Acid	128

2.8	Conclusion and Future Perspectives	131
	References	134
3	Fermentation Technology	149
	<i>Yusuf Chisti</i>	
3.1	Introduction	149
3.2	Types of Fermentations	149
3.3	Fermentation Process	150
3.3.1	Inoculum Generation	151
3.3.2	Growth and Product Formation	152
3.4	Fermentation Medium Design	154
3.5	Sterilization of Air and Fermentation Medium	155
3.6	Environmental Factors	156
3.7	Fermentation Kinetics	157
3.7.1	Batch Fermentation	157
3.7.2	Continuous Culture	158
3.8	Fermentation Equipment	159
3.8.1	Submerged Fermentation	159
3.8.1.1	Stirred Tank Fermenter	159
3.8.1.2	Bubble Column	159
3.8.1.3	Airlift Fermenters	160
3.8.1.4	Fluidized Bed Fermenter	160
3.8.1.5	Trickle-Bed Fermenter	161
3.8.2	Solid-State Fermentation	165
3.8.2.1	Tray Fermenter	165
3.8.2.2	Static Bed Fermenter	167
3.8.2.3	Tunnel Fermenter	167
3.8.2.4	Rotary Disk Fermenter	168
3.8.2.5	Rotary Drum Fermenter	168
3.8.2.6	Agitated Tank Fermenter	168
3.8.2.7	Continuous Screw Fermenter	168
3.9	Recovery of Fermentation Products	169
3.10	Concluding Remarks	170
	References	170
4	Directed Evolution of Industrial Biocatalysts	173
	<i>Marlen Schmidt, Dominique Böttcher, and Uwe T. Bornscheuer</i>	
4.1	Introduction	173
4.2	Strategies for Protein Design	174
4.2.1	Rational Protein Design	174
4.2.2	Directed Evolution	175
4.2.2.1	Mutagenesis Methods	175
4.2.3	Focused Directed Evolution	178
4.3	Assay Systems	179
4.3.1	Selection	180

4.3.1.1	Display Techniques	180
4.3.1.2	<i>In Vivo</i> Selection	181
4.3.2	Screening	182
4.3.2.1	Hydrolase Assays	182
4.3.2.2	Oxidoreductase Assays	184
4.3.2.3	Hydroxynitrile Lyase Assays	185
4.4	Examples	186
4.4.1	Esterases, Lipases, and Phospholipases	186
4.4.2	Nitrilase	188
4.4.3	Halohydrin Dehalogenase	189
4.4.4	P450 Monooxygenase	190
4.4.5	Aldolases	190
4.5	Conclusions	191
	References	197
5	The Industrial Production of Enzymes	207
	<i>Reeta Rani Singhania, Anil Kumar Patel, and Ashok Pandey</i>	
5.1	Introduction	207
5.2	Enzyme Production	208
5.2.1	Selection of a Suitable Enzyme	209
5.2.2	Selection of a Suitable Production Strain	209
5.2.3	Production Methodology	210
5.2.3.1	Submerged Fermentation	210
5.2.3.2	Solid-State Fermentation	211
5.2.4	Downstream Processing	212
5.3	Enzyme Improvement	213
5.3.1	Recombinant DNA Technology	214
5.3.2	Protein Engineering	215
5.4	Large-Scale Enzyme Applications	216
5.4.1	Detergents	216
5.4.2	Food Industry	218
5.4.2.1	Baking	218
5.4.2.2	Starch Hydrolysis and Fructose Production	218
5.4.2.3	Drinks and Dairy	219
5.4.3	Animal Feed	219
5.4.4	Textiles	220
5.4.5	Pulp and Paper	220
5.4.6	Leather	221
5.4.7	Biofuel from Biomass	221
5.4.8	Enzyme Applications in the Chemistry and Pharma Sectors	221
5.4.9	Specialty Enzymes	222
5.4.10	Enzymes in Personal Care Products	222
5.4.11	Enzymes in DNA Technology	222
5.5	Conclusions	223
	References	224

6	Applied Biocatalysis: An Overview	227
	<i>Pedro Fernandes and Joaquim M.S. Cabral</i>	
6.1	Introduction	227
6.2	The Design of the Bioconversion System	229
6.2.1	The Biocatalyst	229
6.2.1.1	Selection	229
6.2.1.2	Whole Cells or Isolated Enzymes?	230
6.2.1.3	Immobilization of Biocatalysts	232
6.2.2	The Bioconversion Medium	237
6.2.2.1	Organic Solvents	237
6.2.2.2	Ionic Liquids	240
6.2.2.3	Two-Phase Aqueous Systems	241
6.2.2.4	Solid Resins	241
6.2.2.5	Solid–Gas Systems	242
6.2.2.6	Supercritical Fluids	242
6.3	Bioreactors	243
6.3.1	Batch Reactors	243
6.3.2	Fed-Batch Reactors	243
6.3.3	Continuous Reactors	244
6.4	Rationalizing and Speeding up the Development and Characterization of a Bioconversion Process	244
6.4.1	Computational Methods	245
6.4.2	Microscale Processing Methods	245
6.5	Concluding Remarks	246
	Acknowledgments	247
	References	247
7	Nanobiotechnology	251
	<i>Rudy J. Koopmans</i>	
7.1	Setting the Stage	251
7.2	Industrial Perspective	252
7.3	Nanotechnology in Biology and Biochemistry	253
7.4	Biomimicry	254
7.4.1	Silk Fibers	255
7.4.2	Gecko Adhesives	257
7.4.3	Nacre and Biomineralization	258
7.5	Materials and Products	259
7.5.1	Peptides and Proteins	259
7.5.1.1	Self-Assembling Peptide and Protein Applications	260
7.5.1.2	Antimicrobial Peptides	261
7.5.1.3	Antifreeze Proteins	261
7.5.1.4	Biosynthetic Hybrids	262
7.5.2	Polynucleotides	263
7.5.3	Lipids	263
7.5.4	Carbohydrates	265

7.6	Processes and Devices	266
7.6.1	Nanomachines	266
7.6.2	Biosensors and Biochips	269
7.6.3	Bioreactors	269
7.6.4	Catalysts	270
7.6.5	Scaffolds	271
7.7	Outlook	271
	References	272
8	Downstream Processing in Industrial Biotechnology	279
	<i>Rajni Hatti-Kaul</i>	
8.1	Introduction	279
8.2	Separations in Industrial Biotechnology	281
8.2.1	Separation of Solid Particles	281
8.2.1.1	Filtration	281
8.2.1.2	Microfiltration	283
8.2.1.3	Centrifugation	284
8.2.1.4	Hydrocyclone	286
8.2.1.5	Flotation and Extraction	286
8.2.2	Cell Disruption for Release of Cell-Associated Products	287
8.2.3	Size-Based Separation of Molecules	288
8.2.3.1	Membrane Filtration	288
8.2.3.2	Size Exclusion Chromatography	292
8.2.4	Separations Based on Product Volatility	292
8.2.4.1	Distillation	293
8.2.4.2	Gas Stripping	293
8.2.4.3	Membrane Distillation and Pervaporation	293
8.2.5	Separations Based on Product Solubility	295
8.2.5.1	Extraction	295
8.2.5.2	Precipitation and Crystallization	299
8.2.6	Molecular Separations Based on Adsorption to a Solid Matrix	301
8.2.6.1	Adsorption Chromatography	304
8.2.6.2	Continuous Chromatography	307
8.2.7	Molecularly Imprinted Materials for Selective Product Capture	309
8.2.8	Membrane Separation of Ionic Solutes: Electrodialysis	310
8.2.9	Chiral Separations Using Membranes	310
8.2.10	Drying/Solvent Removal	311
8.3	Examples of Downstream Processing of Different Product Groups	312
8.3.1	Alcohols	312
8.3.2	Organic Acids	313
8.3.3	Amino Acids	315
8.3.4	Enzymes and Proteins/Peptides	316
8.3.5	Antibiotics	317
8.3.6	Carotenoids	317
8.3.7	Biosurfactants	318

- 8.3.8 Polyhydroxyalkanoates 318
- Acknowledgments 318
- References 319

9 Industrial Biotechnology in the Chemical and Pharmaceutical Industries 323

Maurice C.R. Franssen, Manfred Kircher, and Roland Wohlgemuth

- 9.1 Introduction 323
- 9.2 Biocatalytic Processes: Scientific and Technological Perspectives 324
 - 9.2.1 Beta-Lactam Antibiotics 324
 - 9.2.1.1 The Nucleus 324
 - 9.2.1.2 The Side-Chains 325
 - 9.2.1.3 Enzymatic Semi-Synthesis 327
 - 9.2.2 Chiral Building Blocks 327
 - 9.2.3 Building Blocks for Polymers 328
 - 9.2.4 Fine Chemicals: Statins 330
 - 9.2.5 Amino Acids 334
 - 9.2.6 Indigotin 336
- 9.3 Biocatalytic Processes: Business and Commercial Perspective 338
- 9.4 Safety, Health, and the Environmental Perspective 341
- 9.5 Outlook 342
- Acknowledgments 348
- References 348

10 Industrial Biotechnology in the Food and Feed Sector 351

Pierre Monsan and Michael J. O'Donohue

- 10.1 Introduction 351
- 10.2 Food Applications 352
 - 10.2.1 Starch Transformation 352
 - 10.2.2 Dairy Industry 353
 - 10.2.2.1 Milk-Clotting Enzymes 354
 - 10.2.2.2 Cheese Ripening and Flavor 355
 - 10.2.2.3 Lipase 355
 - 10.2.2.4 Proteases 355
 - 10.2.2.5 Lysozyme 355
 - 10.2.2.6 Transglutaminase 355
 - 10.2.2.7 β -Galactosidase 355
 - 10.2.3 Baking Industry 356
 - 10.2.3.1 Amylases 356
 - 10.2.3.2 Xylanases 357
 - 10.2.3.3 Oxidases 357
 - 10.2.3.4 Phospholipase 358
 - 10.2.3.5 Dextranucrase 358
- 10.2.4 Beer-Making Industry 358

10.2.4.1	Malting	358
10.2.4.2	Prevention of Chill Haze in Beer	359
10.2.5	Fruit Processing	359
10.2.5.1	Pectinases	359
10.2.5.2	Enzymatic Maceration of Fruit	360
10.2.5.3	Grapes and the Wine Industry	361
10.3	Food and Feed Applications	361
10.3.1	Probiotics	361
10.3.2	Prebiotics	363
10.3.2.1	Inulin	363
10.3.2.2	Fructooligosaccharides	364
10.3.2.3	Galactooligosaccharides	364
10.3.2.4	Glucooligosaccharides	366
10.3.2.5	Resistant Starch	368
10.4	Feed Applications	370
10.4.1	Phytate Hydrolysis	370
10.4.2	Carbohydrate Hydrolysis	372
10.4.3	Amino Acid Production	373
	References	374
11	Industrial Biotechnology in the Paper and Pulp Sector	385
	<i>Liisa Viikari, Stina Grönqvist, Kristiina Kruus, Jaakko Pere, Matti Siika-aho, and Anna Suurnäkki</i>	
11.1	Introduction	385
11.2	Enzymes for the Pulp and Paper Industry	387
11.2.1	Cellulases	388
11.2.2	Hemicellulases	389
11.2.3	Transferases	390
11.2.4	Lignin-Modifying, Oxidative Enzymes	390
11.2.4.1	Peroxidases	391
11.2.4.2	Laccases	392
11.3	Enzymes as Process Aids	392
11.3.1	Pulping	393
11.3.1.1	Chemical Pulping	393
11.3.1.2	Mechanical Pulping	395
11.3.2	Bleaching	396
11.3.2.1	Xylanase-Aided Bleaching	397
11.3.2.2	Delignifying Laccase-Mediator System for Direct Bleaching	398
11.3.3	Paper Making	399
11.3.4	Recycled Paper and Deinking	400
11.3.5	Slime Control	401
11.3.6	Other Applications	401
11.4	Enzymes for Product Design	401
11.4.1	Enzymatic Fiber Engineering	401
11.4.2	Functionalized, Value-Added Fibers	402

11.5	Biorefinery Concepts	403
11.6	Conclusions	404
	References	405
12	Biofuels: Production and Applications	413
	<i>Alexandre Rojey and Frédéric Monot</i>	
12.1	A Renewed Interest in Biofuels	413
12.2	Present Conversion Pathways	413
12.3	Biodiesel Production from Vegetable Oils and Fats	416
12.3.1	Esterification Processes	416
12.3.2	Properties of Vegetable Oil Esters	417
12.3.3	Hydrogenation Processes	418
12.4	Ethanol and ETBE Production	419
12.4.1	Ethanol Production from Sugar and Starch	419
12.4.2	ETBE Production	420
12.4.3	Properties of Ethanol and ETBE	420
12.5	The Need for New Developments	421
12.6	Lignocellulosic Biomass Resources	422
12.7	Production of Ethanol from Lignocellulosic Biomass	422
12.7.1	Overall Conversion Scheme	422
12.7.2	Biomass Pretreatment	423
12.7.3	Enzymatic Hydrolysis	424
12.7.4	Fermentation of Glucose and Pentoses	425
12.8	Production of Biofuels Through the Thermochemical Pathway	425
12.8.1	Present Status	425
12.8.2	Pyrolysis and Torrefaction	426
12.8.3	Production of Synthesis Motor Fuels from Biomass	427
12.9	Biorefineries	428
12.10	Biofuels and Sustainability	429
12.11	Conclusion	430
	References	431
13	Environmental and Economic Aspects of Industrial Biotechnology	433
	<i>Barbara G. Hermann, Veronika Dornburg, and Martin K. Patel</i>	
13.1	Introduction	433
13.2	Methodology	434
13.2.1	Generic Approach	434
13.2.1.1	Methodological Background	434
13.2.1.2	Process Design of Industrial Biotechnology Routes	435
13.2.1.3	Technology Assumptions for Industrial Biotechnology Routes	435
13.2.1.4	Energy Use	438
13.2.2	Environmental Impacts Methodology	438
13.2.2.1	System Boundaries	439
13.2.2.2	Allocation and System Expansion	440
13.2.2.3	Production of Fermentable Sugar	441

13.2.2.4	Life Cycle Inventory	441
13.2.2.5	Environmental Indicators	441
13.2.3	Process Economics	442
13.2.3.1	Prices of Fermentable Sugars	444
13.2.3.2	Prices of Utilities and Auxiliaries	444
13.3	Overall Results	444
13.3.1	Results of Environmental Analysis	444
13.3.2	Results of Economic Analysis	447
13.4	Conclusions	451
	References	452
14	Societal Issues in Industrial Biotechnology	457
	<i>Patricia Osseweijer, Klaus Ammann, and Julian Kinderlerer</i>	
14.1	Introduction	457
14.2	The Impact of Industrial Biotechnology	459
14.2.1	How Does This Influence Our Society?	459
14.2.2	What Are the Political, Industrial, Economic, and Scientific Drivers and Obstacles?	460
14.3	Public Perceptions of Industrial Biotechnology	461
14.3.1	What Is the Present Public Perception of Industrial Biotechnology?	462
14.3.2	What Is the Impact of Public Perception to Policy Development?	463
14.3.3	Industrial Reactions to Labeling of GM Food Products	465
14.3.4	Development of Public Interaction	467
14.4	Societal Issues in Industrial Biotechnology	469
14.4.1	Criteria for Communication	469
14.4.2	Novel Approaches to Communication	471
14.4.3	Three International Workshops Identifying Future Issues in Industrial Biotechnology: A Case Study	472
14.4.4	Further Analysis of the Identified Societal Issues Related to Industrial Biotechnology	474
14.4.5	Other Relevant Studies and Committee Reports	478
14.5	Conclusions and Discussion: A Joint Agenda for the Smooth Introduction of Acceptable Sustainable Industrial Biotechnology	479
14.5.1	Hurdles and Challenges	480
14.5.2	Recommendations for Further Studies	480
14.5.3	What Does It Mean for Citizens?	480
	Acknowledgement	481
	References	481