

Contents

Preface to the Second Edition	<i>XV</i>
Preface to the First German Edition	<i>XVII</i>
Preface to the First English Edition	<i>XX</i>

1	Introduction to Enzyme Technology	<i>1</i>
1.1	Introduction	<i>1</i>
1.1.1	What are Biocatalysts?	<i>2</i>
1.1.2	Bio- and Chemocatalysts – Similarities and Differences	<i>2</i>
1.2	Goals and Potential of Biotechnological Production Processes	<i>4</i>
1.3	Historical Highlights of Enzyme Technology/Applied Biocatalysis	<i>8</i>
1.3.1	Early Developments	<i>8</i>
1.3.2	Scientific Progress Since 1890: The Biochemical Paradigm; Growing Success in Application	<i>10</i>
1.3.3	Developments Since 1950	<i>13</i>
1.4	Biotechnological Processes: The Use of Isolated or Intracellular Enzymes as Biocatalysts	<i>15</i>
1.5	Advantages and Disadvantages of Enzyme-Based Production Processes	<i>19</i>
1.6	Goals and Essential System Properties for New or Improved Enzyme Processes	<i>22</i>
1.6.1	Goals	<i>22</i>
1.6.2	Essential System Properties for Rational Design of an Enzyme Process	<i>24</i>
1.6.3	Current Use and Potential of Enzyme Technology	<i>27</i>
	Exercises	<i>28</i>
	Literature	<i>29</i>
	References	<i>29</i>
2	Basics of Enzymes as Biocatalysts	<i>33</i>
2.1	Introduction	<i>34</i>
2.2	Enzyme Classification	<i>35</i>
2.3	Enzyme Synthesis and Structure	<i>37</i>
2.4	Enzyme Function and Its General Mechanism	<i>41</i>

2.5	Free Energy Changes and the Specificity of Enzyme-Catalyzed Reactions	52
2.6	Equilibrium- and Kinetically Controlled Reactions Catalyzed by Enzymes	54
2.7	Kinetics of Enzyme-Catalyzed Reactions	58
2.7.1	Quantitative Relations for Kinetic Characteristics and Selectivities of Enzyme-Catalyzed Reactions	59
2.7.1.1	Turnover Number (k_{cat}) and Michaelis–Menten Constant (K_m)	59
2.7.1.2	Stereoselectivities for Equilibrium- and Kinetically Controlled Reactions	63
2.7.2	Dependence of k_{cat} , K_m , and Selectivities on pH, Temperature, Inhibitors, Activators, and Ionic Strength in Aqueous Solutions	68
2.7.2.1	pH Dependence	69
2.7.2.2	Temperature Dependence	71
2.7.2.3	Binding of Activator and Inhibitor Molecules	73
2.7.2.4	Influence of Ionic Strength	75
2.8	End Points of Enzyme Processes and Amount of Enzyme Required to Reach the End Point in a Given Time	76
2.8.1	Temperature Dependence of the Product Yield	79
2.8.2	pH Dependence of the Yield at the End Point	79
2.8.3	End Points for Kinetic Resolutions of Racemates	82
2.9	Enzyme-Catalyzed Processes with Slightly Soluble Products and Substrates	83
2.9.1	Enzyme-Catalyzed Processes in Aqueous Suspensions	84
2.9.1.1	Changes in Rates, k_{cat} , K_m , and Selectivities in These Systems Compared with Homogeneous Aqueous Solutions	85
2.9.2	Enzyme-Catalyzed Processes in Nonconventional Solvents Where Products and Substrates Are Dissolved (and the Enzyme Suspended)	85
2.9.2.1	Changes in Rates, k_{cat} , K_m , and Selectivities in These Systems Compared with Homogeneous Aqueous Solutions	89
2.10	Stability, Denaturation, and Renaturation of Enzymes	91
2.11	Better Enzymes by Natural Evolution, <i>In Vitro</i> Evolution, or Rational Enzyme Engineering	94
2.11.1	Changes in Enzyme Properties by Natural Evolution	96
2.11.1.1	k_{cat} and K_m	96
2.11.1.2	Enzyme Stability	99
2.11.1.3	Stereoselectivity	100
2.11.1.4	Selectivity in Kinetically Controlled Synthesis of Condensation Products	100
	Exercises	101
	Literature	105
	References	106

3	Enzyme Discovery and Protein Engineering	111
3.1	Enzyme Discovery	111
3.2	Strategies for Protein Engineering	115
3.2.1	Rational Protein Design	117
3.2.2	Directed (Molecular) Evolution	118
3.2.2.1	Methods to Create Mutant Libraries	118
3.2.2.2	Assay Systems	121
3.2.2.3	Examples	124
3.2.3	Focused Directed Evolution	129
3.3	Computational Design of Enzymes	131
	Exercises	132
	References	132
4	Enzymes in Organic Chemistry	141
4.1	Introduction	141
4.1.1	Kinetic Resolution or Asymmetric Synthesis	143
4.2	Examples	144
4.2.1	Oxidoreductases (EC 1)	144
4.2.1.1	Dehydrogenases (EC 1.1.1.-, EC 1.2.1.-, EC 1.4.1.-)	144
4.2.1.2	Oxygenases	148
4.2.1.3	Peroxidases (EC 1.11.1.10)	157
4.2.1.4	Enoate Reductases (EC 1.4.1.31)	157
4.2.1.5	Monoamine Oxidases	159
4.2.2	Transaminases	161
4.2.3	Hydrolases (EC 3.1)	165
4.2.3.1	Lipases (EC 3.1.1.3)	165
4.2.3.2	Esterases (EC 3.1.1.1)	172
4.2.3.3	Peptidases, Acylases, and Amidases	176
4.2.3.4	Epoxide Hydrolases (EC 3.3.2.3)	178
4.2.3.5	Dehalogenases (EC 3.8.1.5)	182
4.2.3.6	Nitrilases (EC 3.5.5.1) and Nitrile Hydratases (EC 4.2.1.84)	182
4.2.3.7	Hydantoinases (EC 3.5.2.-)	187
4.2.4	Lyases (EC 4)	189
4.2.4.1	Hydroxynitrile Lyases (EC 4.1.2.-)	189
4.2.4.2	Aldolases (EC 4.1.2.-, EC 4.1.3.-)	193
4.2.5	Isomerases (EC 5)	197
	Exercises	199
	Literature	199
	References	199
5	Cells Designed by Metabolic Engineering as Biocatalysts for Multienzyme Biotransformations	209
5.1	Introduction	209
5.2	A Short Introduction to Metabolic Engineering	210
5.3	Examples	214

5.3.1	1,3-Propanediol	214
5.3.2	Synthesis of “Biodiesel” and Other Fatty Acid Derivatives	215
5.3.3	Conversion of Cellulosics to Ethanol	216
5.3.4	Conversion of D-Fructose to D-Mannitol	218
5.3.5	Synthesis of L-Ascorbic Acid	219
5.3.6	Other Examples	220
	Exercises	222
	Literature	222
	References	222
6	Enzyme Production and Purification	225
6.1	Introduction	226
6.2	Enzyme Sources	227
6.2.1	Animal and Plant Tissues	227
6.2.2	Wild-Type Microorganisms	229
6.2.3	Recombinant Microorganisms	231
6.3	Improving Enzyme Yield	231
6.3.1	Processes that Influence the Enzyme Yield	233
6.4	Increasing the Yield of Periplasmic and Extracellular Enzymes	236
6.4.1	Penicillin Amidase	238
6.4.2	Lipase	243
6.5	Downstream Processing of Enzymes	245
6.5.1	Static and Dynamic Properties of Chromatographic Adsorbents that Must Be Known for a Rational Design of Chromatographic Protein Purification	249
6.5.1.1	Static Properties	249
6.5.1.2	Dynamic Properties	252
6.5.2	Chromatographic Purification of Enzymes: Problems and Procedures	255
6.5.2.1	Problems	255
6.5.2.2	Procedures	255
6.5.3	Chromatographic Purification and Conditioning of Technical and Therapeutic Enzymes	257
6.5.3.1	Technical Enzymes	257
6.5.3.2	Enzymes for Therapy and Diagnostics	259
6.6	Regulations Based on Risk Assessments/Safety Criteria that Influence the Production of Enzymes and Their Use for Analytical, Pharmaceutical, Scientific, and Technical Purposes	260
6.6.1	Regulations Governing the Use of Genetically Modified Microorganisms for the Production of Enzymes in Laboratories and Production Facilities	260
6.6.2	Regulations Governing the Use of Enzymes Produced in Wild-Type or Recombinant Organisms	265

Exercises	267
Literature	268
References	269

7 Application of Enzymes in Solution: Soluble Enzymes and Enzyme Systems 275

7.1	Introduction and Areas of Application	276
7.1.1	The Impact of Genetic Engineering	278
7.1.2	Medium Design	279
7.1.3	Safety Aspects	280
7.2	Space–Time Yield and Productivity	281
7.3	Examples for the Application of Enzymes in Solution	286
7.3.1	Survey	286
7.3.1.1	Food Applications	289
7.3.1.2	Other Industrial Applications	290
7.3.2	Starch Processing	291
7.3.3	Detergents	294
7.4	Membrane Systems and Processes	297
	Exercises	305
	Literature	307
	References	308

8 Immobilization of Enzymes (Including Applications) 313

8.1	Principles	313
8.1.1	Parameters of Immobilization	317
8.2	Carriers	319
8.2.1	Inorganic Carriers	321
8.2.2	Polysaccharides	321
8.2.3	Synthetic Polymers	326
8.3	Binding Methods	330
8.3.1	Adsorption	330
8.3.2	Covalent Binding	331
8.4	Examples: Application of Immobilized Enzymes	335
8.4.1	Hydrolysis and Biotransformation of Carbohydrates	335
8.4.2	Hydrolysis and Synthesis of Penicillins and Cephalosporins	345
8.4.3	Further Processes	346
8.4.3.1	Amino Acid, Peptide, and Amide Synthesis	346
8.4.3.2	Application of Lipases	348
	Exercises	349
	Literature	352
	References	352

9 Immobilization of Microorganisms and Cells 359

9.1	Introduction	359
9.2	Fundamental Aspects	362

9.3	Immobilization by Aggregation/Flocculation	364
9.4	Immobilization by Entrapment	368
9.4.1	Entrapment in Polymeric Networks	368
9.4.2	Entrapment in Ionotropic Gels	369
9.4.2.1	Principle	369
9.4.2.2	Examples	372
9.5	Adsorption	375
9.6	Adhesion	376
9.6.1	Basic Considerations	377
9.6.2	Applications	383
9.6.2.1	Adherent Mammalian Cells for Biopharmaceuticals Production	383
9.6.2.2	Anaerobic Wastewater Treatment	384
9.6.2.3	Nitrogen Elimination (Nitrification and Denitrification)	391
9.6.2.4	Exhaust Gas Purification	391
9.7	Perspectives	393
9.7.1	Biofilm Catalysis	393
9.7.2	Microbial Fuel Cells	396
	Exercises	399
	References	402
10	Characterization of Immobilized Biocatalysts	411
10.1	Introduction	412
10.2	Factors Influencing the Space–Time Yield of Immobilized Biocatalysts	413
10.3	Effectiveness Factors for Immobilized Biocatalysts	414
10.4	Mass Transfer and Reaction	416
10.4.1	Maximal Reaction Rate of Immobilized Biocatalysts as a Function of Particle Radius	416
10.4.2	Calculation of Effectiveness Factors and Concentration Profiles Inside and Outside the Particles	418
10.5	Space–Time Yields and Effectiveness Factors for Different Reactors	422
10.5.1	Continuous Stirred Tank Reactor	423
10.5.2	Packed Bed Reactor or Stirred Batch Reactor	424
10.5.3	Comparison of CST and PB Reactors	425
10.6	Determination of Essential Properties of Immobilized Biocatalysts	425
10.6.1	Physicochemical Properties	428
10.6.1.1	Immobilized Biocatalyst Distribution and Conformation	429
10.6.1.2	Stationary Charge Density in the Support	429
10.6.2	Kinetic Characterization of Immobilized Biocatalysts: Influence of Support Properties on the Nano- and Micrometer Level in Aqueous and Other Systems	430
10.6.2.1	Determination of V'_{\max} , k'_{cat} , Substrate/Product Concentration, and pH Gradients	431

- 10.6.2.2 K'_m and K'_i 434
- 10.6.2.3 Selectivities 435
- 10.6.2.4 Determinations of Effectiveness Factors 436
- 10.6.3 Productivity and Stability under Process Conditions 436
- 10.7 Comparison of Calculated and Experimental Data for Immobilized Biocatalysts 437
- 10.8 Application of Immobilized Biocatalysts for Enzyme Processes in Aqueous Suspensions 439
- 10.9 Improving the Performance of Immobilized Biocatalysts 441
 - Exercises 443
 - References 445

- 11 Reactors and Process Technology 449**
 - 11.1 General Aspects, Biochemical Engineering, and Process Sustainability 449
 - 11.1.1 Biochemical Engineering Aspects 450
 - 11.1.2 Process Sustainability and Ecological Considerations 452
 - 11.2 Types of Reactors 454
 - 11.2.1 Basic Types and Mass Balances 455
 - 11.2.2 Other Reactor Types and Configurations: Application Examples 460
 - 11.3 Residence Time Distribution, Mixing, Pressure Drop, and Mass Transfer in Reactors 466
 - 11.3.1 Scale-Up, Dimensionless Numbers 466
 - 11.3.2 Residence Time Distribution 468
 - 11.3.3 Mixing in Stirred Tank Reactors 471
 - 11.3.4 Mass Transfer in Reactors 476
 - 11.3.5 Pressure Drop and Fluidization in Tubular Reactors 477
 - 11.4 Process Technology 478
 - 11.4.1 Survey 478
 - 11.4.2 Process Integration 479
 - 11.4.3 Reactor Instrumentation 486
 - Exercises 486
 - Literature 488
 - References 488

- 12 Case Studies 493**
 - 12.1 Starch Processing and Glucose Isomerization 493
 - 12.1.1 Starch Processing 493
 - 12.1.2 The Manufacture of Glucose–Fructose Syrup 497
 - 12.2 Biofuels from Biomass 501
 - 12.2.1 Starch-Based Ethanol Production 502
 - 12.2.2 Lignocellulose-Based Biofuels 507
 - 12.2.2.1 General 507
 - 12.2.2.2 Raw Materials 508
 - 12.2.2.3 Pretreatment 509

12.2.2.4	Enzymes	512
12.2.2.5	Processing and Reaction Engineering	517
12.2.2.6	Pilot Studies	519
12.2.2.7	Alternative Biocatalyst-Based Biofuels	520
12.3	Case Study: the One-Step Enzymatic Process to Produce 7-ACA from Cephalosporin C	521
12.3.1	Enzyme Processes for the Production of β -Lactam Antibiotics	521
12.3.2	Overall Process for the Production of 7-ACA	531
12.3.3	Conversion of Cephalosporin C to 7-ACA	533
12.3.4	Reaction Characterization and Identification of Constraints: Hydrolysis of Cephalosporin C	533
12.3.5	Enzyme Characterization and Identification of Constraints: Cephalosporin Acylase (or Glutaryl Acylase or Amidase)	535
12.3.6	Evaluation of Process Options	536
12.3.6.1	Process Window	536
12.3.6.2	Suitable Reactors and pH-Controlling Buffers	537
12.3.6.3	Reaction End Point and Immobilized Enzyme Requirement for Minimum Space–Time Yield	539
12.3.6.4	Product Isolation	539
12.4	Case Study: Biocatalytic Process for the Synthesis of the Lipitor Side Chain	540
	Exercises	543
	References	544

Appendix A: The World of Biotechnology Information: Seven Points for Reflecting on Your Information Behavior 553

Appendix B: Solutions to Exercises 565

Appendix C: Symbols and Abbreviations 585

Index 591