

Contents

Preface V

Acknowledgments VII

1	Introduction to Biocatalysis	1
1.1	Overview: The Status of Biocatalysis at the Turn of the 21st Century	2
1.1.1	State of Acceptance of Biocatalysis	2
1.1.2	Current Advantages and Drawbacks of Biocatalysis	4
1.1.2.1	Advantages of Biocatalysts	4
1.1.2.2	Drawbacks of Current Biocatalysts	5
1.2	Characteristics of Biocatalysis as a Technology	6
1.2.1	Contributing Disciplines and Areas of Application	6
1.2.2	Characteristics of Biocatalytic Transformations	7
1.2.2.1	Comparison of Biocatalysis with other Kinds of Catalysis	8
1.2.3	Applications of Biocatalysis in Industry	9
1.2.3.1	Chemical Industry of the Future: Environmentally Benign Manufacturing, Green Chemistry, Sustainable Development in the Future	9
1.2.3.2	Enantiomerically Pure Drugs or Advanced Pharmaceutical Intermediates (APIs)	10
1.3	Current Penetration of Biocatalysis	11
1.3.1	The Past: Historical Digest of Enzyme Catalysis	11
1.3.2	The Present: Status of Biocatalytic Processes	11
1.4	The Breadth of Biocatalysis	14
1.4.1	Nomenclature of Enzymes	14
1.4.2	Biocatalysis and Organic Chemistry, or "Do we Need to Forget our Organic Chemistry?"	14
2	Characterization of a (Bio-)catalyst	19
2.1	Characterization of Enzyme Catalysis	20
2.1.1	Basis of the Activity of Enzymes: What is Enzyme Catalysis?	20
2.1.1.1	Enzyme Reaction in a Reaction Coordinate Diagram	21
2.1.2	Development of Enzyme Kinetics from Binding and Catalysis	21

12.3	State of Knowledge of Functioning of Enzymes in Solvents	344
12.3.1	Range of Enzymes, Reactions, and Solvents	344
12.3.2	The Importance of Water in Enzyme Reactions in Organic Solvents	345
12.3.2.1	Exchange of Water Molecules between Enzyme Surface and Bulk Organic Solvent	345
12.3.2.2	Relevance of Water Activity	346
12.3.3	Physical Organic Chemistry of Enzymes in Organic Solvents	347
12.3.3.1	Active Site and Mechanism	347
12.3.3.2	Flexibility of Enzymes in Organic Solvents	347
12.3.3.3	Polarity and Hydrophobicity of Transition State and Binding Site	348
12.3.4	Correlation of Enzyme Performance with Solvent Parameters	349
12.3.4.1	Control through Variation of Hydrophobicity: log P Concept	350
12.3.4.2	Correlation of Enantioselectivity with Solvent Polarity and Hydrophobicity	350
12.4	Optimal Handling of Enzymes in Organic Solvents	351
12.4.1	Enzyme Memory in Organic Solvents	352
12.4.2	Low Activity in Organic Solvents Compared to Water	353
12.4.3	Enhancement of Selectivity of Enzymes in Organic Solvents	354
12.5	Novel Reaction Media for Biocatalytic Transformations	355
12.5.1	Substrate as Solvent (Neat Substrates): Acrylamide from Acrylonitrile with Nitrile Hydratase	355
12.5.2	Supercritical Solvents	356
12.5.3	Ionic Liquids	356
12.5.4	Emulsions [Manufacture of Phosphatidylglycerol (PG)]	357
12.5.5	Microemulsions	358
12.5.6	Liquid Crystals	358
12.5.7	Ice-Water Mixtures	359
12.5.8	High-Density Eutectic Suspensions	361
12.5.9	High-Density Salt Suspensions	362
12.5.10	Solid-to-Solid Syntheses	363
12.6	Solvent as a Parameter for Reaction Optimization ("Medium Engineering")	366
12.6.1	Change of Substrate Specificity with Change of Reaction M: Specificity of Serine Proteases	366
12.6.2	Change of Regioselectivity by Organic Solvent Medium	367
12.6.3	Solvent Control of Enantiospecificity of Nifedipines	367
13	Pharmaceutical Applications of Biocatalysis	373
13.1	Enzyme Inhibition for the Fight against Disease	374
13.1.1	Introduction	374
13.1.2	Procedure for the Development of Pharmacologically Active Compounds	376
13.1.3	Process for the Registration of New Drugs	377
13.1.4	Chiral versus Non-chiral Drugs	379

9.5.3	Lipase, Another Catalytic Triad Mechanism	266
9.5.4	Metalloproteases	268
9.6	Electrophilic catalysis	269
9.6.1	Utilization of Metal Ions: ADH, a Different Catalytic Triad	269
9.6.1.1	Catalytic Mechanism of Horse Liver Alcohol Dehydrogenase, a Medium-Chain Dehydrogenase	269
9.6.1.2	Catalytic Reaction Mechanism of <i>Drosophila</i> ADH, a Short-Chain Dehydrogenase	271
9.6.2	Formation of a Schiff Base, Part I: Acetoacetate Decarboxylase, Aldolase	274
9.6.3	Formation of a Schiff Base with Pyridoxal Phosphate (PLP): Alanine Racemase, Amino Acid Transferase	275
9.6.4	Utilization of Thiamine Pyrophosphate (TPP): Transketolase	277
10	Protein Engineering	281
10.1	Introduction: Elements of Protein Engineering	282
10.2	Methods of Protein Engineering	283
10.2.1	Fusion PCR	284
10.2.2	Kunkel Method	285
10.2.3	Site-Specific Mutagenesis Using the QuikChange Kit from Stratagene	287
10.2.4	Combined Chain Reaction (CCR)	288
10.3	Glucose (Xylose) Isomerase (GI) and Glycoamylase: Enhancement of Thermostability	289
10.3.1	Enhancement of Thermostability in Glucose Isomerase (GI)	289
10.3.2	Resolving the Reaction Mechanism of Glucose Isomerase (GI): Diffusion-Limited Glucose Isomerase?	292
10.4	Enhancement of Stability of Proteases against Oxidation and Thermal Deactivation	293
10.4.1	Enhancement of Oxidation Stability of Subtilisin	293
10.4.2	Thermostability of Subtilisin	295
10.5	Creating New Enzymes with Protein Engineering	295
10.5.1	Redesign of a Lactate Dehydrogenase	295
10.5.2	Synthetic Peroxidases	297
10.6	Dehydrogenases, Changing Cofactor Specificity	298
10.7	Oxygenases	300
10.8	Change of Enantioselectivity with Site-Specific Mutagenesis	302
10.9	Techniques Bridging Different Protein Engineering Techniques	303
10.9.1	Chemically Modified Mutants, a Marriage of Chemical Modification and Protein Engineering	303
10.9.2	Expansion of Substrate Specificity with Protein Engineering and Directed Evolution	304
11	Applications of Recombinant DNA Technology: Directed Evolution	309
11.1	Background of Evolvability of Proteins	310

8.5	Downstream Processing: Concentration and Purification of Proteins	231
8.5.1	Dialysis (Ultrafiltration) (adapted in part from Blanch, 1997)	231
8.5.2	Chromatography	233
8.5.2.1	Theory of Chromatography	233
8.5.2.2	Different Types of Chromatography	235
8.5.3	Drying: Spray Drying, Lyophilization, Stabilization for Storage	236
8.6	Examples of Biocatalyst Purification	237
8.6.1	Example 1: Alcohol Dehydrogenase [(R)-ADH from <i>L. brevis</i> (Riebel, 1997)]	237
8.6.2	Example 2: L-Amino Acid Oxidase from <i>Rhodococcus opacus</i> (Geueke 2002a,b)	238
8.6.3	Example 3: Xylose Isomerase from <i>Thermoanaerobium</i> Strain JW/SL-YS 489	240
9	Methods for the Investigation of Proteins	243
9.1	Relevance of Enzyme Mechanism	244
9.2	Experimental Methods for the Investigation of an Enzyme Mechanism	245
9.2.1	Distribution of Products (Curtin–Hammett Principle)	245
9.2.2	Stationary Methods of Enzyme Kinetics	246
9.2.3	Linear Free Enthalpy Relationships (LFERs): Brønsted and Hammett Effects	248
9.2.4	Kinetic Isotope Effects	249
9.2.5	Non-stationary Methods of Enzyme Kinetics: Titration of Active Sites	249
9.2.5.1	Determination of Concentration of Active Sites	249
9.2.6	Utility of the Elucidation of Mechanism: Transition-State Analog Inhibitors	251
9.3	Methods of Enzyme Determination	253
9.3.1	Quantification of Protein	253
9.3.2	Isoelectric Point Determination	254
9.3.3	Molecular Mass Determination of Protein Monomer: SDS-PAGE	254
9.3.4	Mass of an Oligomeric Protein: Size Exclusion Chromatography (SEC)	256
9.3.5	Mass Determination: Mass Spectrometry (MS) (after Kellner, Lottspeich, Meyer)	257
9.3.6	Determination of Amino Acid Sequence by Tryptic Degradation, or Acid, Chemical or Enzymatic Digestion	258
9.4	Enzymatic Mechanisms: General Acid–Base Catalysis	258
9.4.1	Carbonic Anhydrase II	258
9.4.2	Vanadium Haloperoxidase	260
9.5	Nucleophilic Catalysis	261
9.5.1	Serine Proteases	261
9.5.2	Cysteine in Nucleophilic Attack	265

2.2	Sources and Reasons for the Activity of Enzymes as Catalysts	23
2.2.1	Chronology of the Most Important Theories of Enzyme Activity	23
2.2.2	Origin of Enzymatic Activity: Derivation of the Kurz Equation	24
2.2.3	Consequences of the Kurz Equation	25
2.2.4	Efficiency of Enzyme Catalysis: Beyond Pauling's Postulate	28
2.3	Performance Criteria for Catalysts, Processes, and Process Routes	30
2.3.1	Basic Performance Criteria for a Catalyst: Activity, Selectivity and Stability of Enzymes	30
2.3.1.1	Activity	30
2.3.1.2	Selectivity	31
2.3.1.3	Stability	32
2.3.2	Performance Criteria for the Process	33
2.3.2.1	Product Yield	33
2.3.2.2	(Bio)catalyst Productivity	34
2.3.2.3	(Bio)catalyst Stability	34
2.3.2.4	Reactor Productivity	35
2.3.3	Links between Enzyme Reaction Performance Parameters	36
2.3.3.1	Rate Acceleration	36
2.3.3.2	Ratio between Catalytic Constant k_{cat} and Deactivation Rate Constant k_d	38
2.3.3.3	Relationship between Deactivation Rate Constant k_d and Total Turnover Number TTN	38
2.3.4	Performance Criteria for Process Schemes, Atom Economy, and Environmental Quotient	39
3	Isolation and Preparation of Microorganisms	43
3.1	Introduction	44
3.2	Screening of New Enzyme Activities	46
3.2.1	Growth Rates in Nature	47
3.2.2	Methods in Microbial Ecology	47
3.3	Strain Development	48
3.3.1	Range of Industrial Products from Microorganisms	48
3.3.2	Strain Improvement	50
3.4	Extremophiles	52
3.4.1	Extremophiles in Industry	54
3.5	Rapid Screening of Biocatalysts	56
4	Molecular Biology Tools for Biocatalysis	61
4.1	Molecular Biology Basics: DNA versus Protein Level	62
4.2	DNA Isolation and Purification	65
4.2.1	Quantification of DNA/RNA	66
4.3	Gene Isolation, Detection, and Verification	67
4.3.1	Polymerase Chain Reaction	67
4.3.2	Optimization of a PCR Reaction	69
4.3.3	Special PCR Techniques	71

4.3.3.1	Nested PCR	71
4.3.3.2	Inverse PCR	71
4.3.3.3	RACE: Rapid Amplification of cDNA Ends	71
4.3.4	Southern Blotting	74
4.3.4.1	Probe Design and Labeling	76
4.3.4.2	Hybridization	76
4.3.4.3	Detection	76
4.3.5	DNA-Sequencing	77
4.4	Cloning Techniques	77
4.4.1	Restriction Mapping	78
4.4.2	Vectors	78
4.4.3	Ligation	80
4.4.3.1	Propagation of Plasmids and Transformation in Hosts	81
4.5	(Over)expression of an Enzyme Function in a Host	81
4.5.1	Choice of an Expression System	81
4.5.2	Translation and Codon Usage in <i>E. coli</i>	82
4.5.3	Choice of Vector	84
4.5.3.1	Generation of Inclusion Bodies	85
4.5.3.2	Expression of Fusion Proteins	85
4.5.3.3	Surface Expression	87
4.5.4	Expression of Eukaryotic Genes in Yeasts	87
5	Enzyme Reaction Engineering	91
5.1	Kinetic Modeling: Rationale and Purpose	92
5.2	The Ideal World: Ideal Kinetics and Ideal Reactors	94
5.2.1	The Classic Case: Michaelis–Menten Equation	94
5.2.2	Design of Ideal Reactors	96
5.2.3	Integrated Michaelis–Menten Equation in Ideal Reactors	96
5.2.3.1	Case 1: No Inhibition	97
5.3	Enzymes with Unfavorable Binding: Inhibition	97
5.3.1	Types of Inhibitors	97
5.3.2	Integrated Michaelis–Menten Equation for Substrate and Product Inhibition	99
5.3.2.1	Case 2: Integrated Michaelis–Menten Equation in the Presence of Substrate Inhibitor	99
5.3.2.2	Case 3: Integrated Michaelis–Menten Equation in the Presence of Inhibitor	99
5.3.3	The $K_I - [I]_{50}$ Relationship: Another Useful Application of Mechanism Elucidation	103
5.4	Reactor Engineering	105
5.4.1	Configuration of Enzyme Reactors	105
5.4.1.1	Characteristic Dimensionless Numbers for Reactor Design	107
5.4.2	Immobilized Enzyme Reactor (Fixed-Bed Reactor with Plug-Flow)	108
5.4.2.1	Reactor Design Equations	108
5.4.2.2	Immobilization	109

5.4.2.3	Optimal Conditions for an Immobilized Enzyme Reactor	110
5.4.3	Enzyme Membrane Reactor (Continuous Stirred Tank Reactor, CSTR)	110
5.4.3.1	Design Equation: Reactor Equation and Retention	110
5.4.3.2	Classification of Enzyme Membrane Reactors	111
5.4.4	Rules for Choice of Reaction Parameters and Reactors	113
5.5	Enzyme Reactions with Incomplete Mass Transfer: Influence of Immobilization	113
5.5.1	External Diffusion (Film Diffusion)	114
5.5.2	Internal Diffusion (Pore Diffusion)	114
5.5.3	Methods of Testing for Mass Transfer Limitations	116
5.5.4	Influence of Mass Transfer on the Reaction Parameters	118
5.6	Enzymes with Incomplete Stability: Deactivation Kinetics	119
5.6.1	Resting Stability	119
5.6.2	Operational Stability	120
5.6.3	Comparison of Resting and Operational Stability	122
5.6.4	Strategy for the Addition of Fresh Enzyme to Deactivating Enzyme in Continuous Reactors	124
5.7	Enzymes with Incomplete Selectivity: E-Value and its Optimization	126
5.7.1	Derivation of the E-Value	126
5.7.2	Optimization of Separation of Racemates by Choice of Degree of Conversion	128
5.7.2.1	Optimization of an Irreversible Reaction	128
5.7.2.2	Enantioselectivity of an Equilibrium Reaction	129
5.7.2.3	Determination of Enantiomeric Purity from a Conversion–Time Plot	130
5.7.3	Optimization of Enantiomeric Ratio E by Choice of Temperature	130
5.7.3.1	Derivation of the Isoinversion Temperature	130
5.7.3.2	Example of Optimization of Enantioselectivity by Choice of Temperature	131
6	Applications of Enzymes as Bulk Actives:	
	Detergents, Textiles, Pulp and Paper, Animal Feed	135
6.1	Application of Enzymes in Laundry Detergents	136
6.1.1	Overview	136
6.1.2	Proteases against Blood and Egg Stains	138
6.1.3	Lipases against Grease Stains	139
6.1.4	Amylases against Grass and Starch Dirt	139
6.1.5	Cellulases	139
6.1.6	Bleach Enzymes	140
6.2	Enzymes in the Textile Industry: Stone-washed Denims, Shiny Cotton Surfaces	140
6.2.1	Build-up and Mode of Action of Enzymes for the Textile Industry	140
6.2.2	Cellulases: the Shinier Look	141

6.2.3	Stonewashing: Biostoning of Denim: the Worn Look	143
6.2.4	Peroxidases	144
6.3	Enzymes in the Pulp and Paper Industry: Bleaching of Pulp with Xylanases or Laccases	145
6.3.1	Introduction	145
6.3.2	Wood	146
6.3.2.1	Cellulose	146
6.3.2.2	Hemicellulose	147
6.3.2.3	Lignin	147
6.3.3	Papermaking: Kraft Pulping Process	149
6.3.4	Research on Enzymes in the Pulp and Paper Industry	150
6.3.4.1	Laccases	150
6.3.4.2	Xylanases	151
6.3.4.3	Cellulases in the Papermaking Process	152
6.4	Phytase for Animal Feed: Utilization of Phosphorus	152
6.4.1	The Farm Animal Business and the Environment	152
6.4.2	Phytase	153
6.4.3	Efficacy of Phytase: Reduction of Phosphorus	154
6.4.4	Efficacy of Phytase: Effect on Other Nutrients	155
7	Application of Enzymes as Catalysts: Basic Chemicals, Fine Chemicals, Food, Crop Protection, Bulk Pharmaceuticals	159
7.1	Enzymes as Catalysts in Processes towards Basic Chemicals	160
7.1.1	Nitrile Hydratase: Acrylamide from Acrylonitrile, Nicotinamide from 3-Cyanopyridine, and 5-Cyanovaleramide from Adiponitrile	160
7.1.1.1	Acrylamide from Acrylonitrile	160
7.1.1.2	Nicotinamide from 3-Cyanopyridine	162
7.1.1.3	5-Cyanovaleramide from Adiponitrile	162
7.1.2	Nitrilase: 1,5-Dimethyl-2-piperidone from 2-Methylglutaronitrile	163
7.1.3	Toluene Dioxygenase: Indigo or Prostaglandins from Substituted Benzenes via cis-Dihydrodiols	163
7.1.4	Oxynitrilase (Hydroxy Nitrile Lyase, HNL): Cyanohydrins from Aldehydes	167
7.2	Enzymes as Catalysts in the Fine Chemicals Industry	170
7.2.1	Chirality, and the Cahn–Ingold–Prelog and Pfeiffer Rules	170
7.2.2	Enantiomerically Pure Amino Acids	172
7.2.2.1	The Aminoacylase Process	172
7.2.2.2	The Amidase Process	174
7.2.2.3	The Hydantoinase/Carbamoylase Process	174
7.2.2.4	Reductive Amination of Keto Acids (L-tert-Leucine as Example)	177
7.2.2.5	Aspartase	180
7.2.2.6	L-Aspartate- β -decarboxylase	180
7.2.2.7	L-2-Aminobutyric acid	181
7.2.3	Enantiomerically Pure Hydroxy Acids, Alcohols, and Amines	182
7.2.3.1	Fumarase	182

7.2.3.2	Enantiomerically Pure Amines with Lipase	182
7.2.3.3	Synthesis of Enantiomerically Pure Amines through Transamination	183
7.2.3.4	Hydroxy esters with carbonyl reductases	185
7.2.3.5	Alcohols with ADH	186
7.3	Enzymes as Catalysts in the Food Industry	187
7.3.1	HFCS with Glucose Isomerase (GI)	187
7.3.2	Aspartame, Artificial Sweetener through Enzymatic Peptide Synthesis	188
7.3.3	Lactose Hydrolysis	191
7.3.4	"Nutraceuticals": L-Carnitine as a Nutrient for Athletes and Convalescents (Lonza)	191
7.3.5	Decarboxylases for Improving the Taste of Beer	194
7.4	Enzymes as Catalysts towards Crop Protection Chemicals	195
7.4.1	Intermediate for Herbicides: (R)-2-(4-Hydroxyphenoxypropionic acid (BASF, Germany)	195
7.4.2	Applications of Transaminases towards Crop Protection Agents: L-Phosphinothricin and (S)-MOIPA	196
7.5	Enzymes for Large-Scale Pharma Intermediates	197
7.5.1	Penicillin G (or V) Amidase (PGA, PVA): β -Lactam Precursors, Semi-synthetic β -Lactams	197
7.5.2	Ephedrine	200
8	Biotechnological Processing Steps for Enzyme Manufacture	209
8.1	Introduction to Protein Isolation and Purification	210
8.2	Basics of Fermentation	212
8.2.1	Medium Requirements	213
8.2.2	Sterilization	214
8.2.3	Phases of a Fermentation	214
8.2.4	Modeling of a Fermentation	215
8.2.5	Growth Models	216
8.2.6	Fed-Batch Culture	216
8.3	Fermentation and its Main Challenge: Transfer of Oxygen	218
8.3.1	Determination of Required Oxygen Demand of the Cells	218
8.3.2	Calculation of Oxygen Transport in the Fermenter Solution	219
8.3.3	Determination of k_L , a , and $k_L a$	220
8.3.2.1	Methods of Measurement of the Product $k_L a$	221
8.4	Downstream Processing: Crude Purification of Proteins	223
8.4.1	Separation (Centrifugation)	223
8.4.2	Homogenization	225
8.4.3	Precipitation	226
8.4.3.1	Precipitation in Water-Miscible Organic Solvents	228
8.4.3.2	Building Quantitative Models for the Hofmeister Series and Cohn-Edsall and Setschenow Equations	228
8.4.4	Aqueous Two-Phase Extraction	229

14.3.3	Protein Explorer	419
14.3.4	ExPASy Server: Roche Applied Science Biochemical Pathways	419
14.3.5	GenBank	419
14.3.6	SwissProt	420
14.3.7	Information on an Enzyme: the Example of dehydrogenases	420
14.3.7.1	Sequence Information	421
14.3.7.2	Structural Information	422
14.4	Applied Bioinformatics Tools, with Examples	422
14.4.1	BLAST	422
14.4.2	Aligning Several Protein Sequences using ClustalW	425
14.4.3	Task: Whole Genome Analysis	427
14.4.4	Phylogenetic Tree	427
14.5	Bioinformatics for Structural Information on Enzymes	429
14.5.1	The Status of Predicting Protein Three-Dimensional Structure	430
14.6	Conclusion and Outlook	431
15	Systems Biology for Biocatalysis	433
15.1	Introduction to Systems Biology	434
15.1.1	Systems Approach versus Reductionism	434
15.1.2	Completion of Genomes: Man, Earthworm, and Others	435
15.2	Genomics, Proteomics, and other -omics	435
15.2.1	Genomics	435
15.2.2	Proteomics	436
15.3	Technologies for Systems Biology	438
15.3.1	Two-Dimensional Gel Electrophoresis (2D PAGE)	438
15.3.1.1	Separation by Chromatography or Capillary Electrophoresis	439
15.3.1.2	Separation by Chemical Tagging	440
15.3.2	Mass Spectroscopy	441
15.3.2.1	MALDI-TOF-MS (Matrix-Assisted Laser Desorption/Ionization Time-of-Flight MS)	444
15.3.2.2	ESI-triple-quadrupole MS	444
15.3.2.3	ESI-MS Using an Ion Trap Analyzer	445
15.3.3	DNA Microarrays	446
15.3.4	Protein Microarrays	447
15.3.5	Applications of Genomics and Proteomics in Biocatalysis	448
15.3.5.1	Lactic Acid Bacteria and Proteomics	448
15.4	Metabolic Engineering	449
15.4.1	Concepts of Metabolic Engineering	449
15.4.2	Examples of Metabolic Engineering	451
16	Evolution of Biocatalytic Function	457
16.1	Introduction	458
16.1.2	Congruence of Sequence, Function, Structure, and Mechanism	460
16.2	Search Characteristics for Relatedness in Proteins	461
16.2.1	Classification of Relatedness of Proteins: the -log Family	461

16.2.2	Classification into Protein Families	464
16.2.3	Dominance of Different Mechanisms	465
16.3	Evolution of New Function in Nature	466
16.3.1	Dual-Functionality Proteins	469
16.3.1.1	Moonlighting Proteins	469
16.3.1.2	Catalytic Promiscuity	469
16.3.2	Gene Duplication	470
16.3.3	Horizontal Gene Transfer (HGT)	471
16.3.4	Circular Permutation	474
16.4	α/β -Barrel Proteins as a Model for the Investigation of Evolution	474
16.4.1	Why Study α/β -Barrel Proteins?	474
16.4.2	Example of Gene Duplication: Mandelate and α -Ketoadipate Pathways	475
16.4.2.1	Description of Function	480
16.4.3	Exchange of Function in the Aromatic Biosynthesis Pathways: Trp and His Pathways	481
17	Stability of Proteins	487
17.1	Summary: Protein Folding, First-Order Decay, Arrhenius Law	488
17.1.1	The Protein Folding Problem	488
17.1.2	Why do Proteins Fold?	489
17.2	Two-State Model: Thermodynamic Stability of Proteins (Unfolding)	491
17.2.1	Protein Unfolding and Deactivation	491
17.2.2	Thermodynamics of Proteins	491
17.3	Three-State Model: Lumry–Eyring Equation	493
17.3.1	Enzyme Deactivation	493
17.3.2	Empirical Deactivation Model	494
17.4	Four-State Model: Protein Aggregation	496
17.4.1	Folding, Deactivation, and Aggregation	496
17.4.2	Model to Account for Competition between Folding and Inclusion Body Formation	498
17.4.2.1	Case 1: In Vitro – Protein Synthesis Unimportant	498
17.4.2.2	Case 2: In Vivo – Protein Synthesis Included	499
17.5	Causes of Instability of Proteins: $\Delta G < 0$, $\gamma(t)$, A	501
17.5.1	Thermal Inactivation	502
17.5.2	Deactivation under the Influence of Stirring	503
17.5.3	Deactivation under the Influence of Gas Bubbles	504
17.5.4	Deactivation under the Influence of Aqueous/Organic Interfaces	505
17.5.5	Deactivation under the Influence of Salts and Solvents	505
17.6	Biotechnological Relevance of Protein Folding: Inclusion Bodies	505
17.7	Summary: Stabilization of Proteins	506
17.7.1	Correlation between Stability and Structure	507

18	Artificial Enzymes	511
18.1	Catalytic Antibodies	512
18.1.1	Principle of Catalytic Antibodies: Connection between Chemistry and Immunology	512
18.1.2	Test Reaction Selection, Haptens, Mechanisms, Stabilization	514
18.1.2.1	Mechanism of Antibody-Catalyzed Reactions	516
18.1.2.2	Stabilization of Charged Transition States	517
18.1.2.3	Effect of Antibodies as Entropy Traps	517
18.1.3	Breadth of Reactions Catalyzed by Antibodies	518
18.1.3.1	Fastest Antibody-Catalyzed Reaction in Comparison with Enzymes	518
18.1.3.2	Antibody-Catalyzed Reactions without Corresponding Enzyme Equivalent	518
18.1.3.3	Example of a Pericyclic Reaction: Claisen Rearrangement	518
18.1.3.4	Antibody Catalysts with Dual Activities	518
18.1.3.5	Scale-Up of an Antibody-Catalyzed Reaction	520
18.1.3.6	Perspective for Catalytic Antibodies	520
18.2	Other Proteinaceous Catalysts: Ribozymes and Enzyme Mimics	521
18.2.1	Ribozymes: RNA World before Protein World?	521
18.2.2	Proteinaceous Enzyme Mimics	521
18.3	Design of Novel Enzyme Activity: Enzyme Models (Synzymes)	523
18.3.1	Introduction	523
18.3.2	Enzyme Models on the Basis of the Binding Step: Diels–Alder Reaction	523
18.3.3	Enzyme Models with Binding and Catalytic Effects	525
18.4	Heterogenized/Immobilized Chiral Chemical Catalysts	526
18.4.1	Overview of Different Approaches	526
18.4.2	Immobilization with Polyamino Acids as Chiral Polymer Catalysts	526
18.4.3	Immobilization on Resins or other Insoluble Carriers	527
18.4.4	Heterogenization with Dendrimers	528
18.4.5	Retention of Heterogenized Chiral Chemical Catalysts in a Membrane Reactor	529
18.4.6	Recovery of Organometallic Catalysts by Phase Change: Liquid–Liquid Extraction	531
18.5	Tandem Enzyme Organometallic Catalysts	532
19	Design of Biocatalytic Processes	539
19.1	Design of Enzyme Processes: High-Fructose Corn Syrup (HFCS)	540
19.1.1	Manufacture of HFCS from Glucose with Glucose Isomerase (GI): Process Details	540
19.1.2	Mathematical Model for the Description of the Enzyme Kinetics of Glucose Isomerase (GI)	541
19.1.3	Evaluation of the Model of the GI Reaction in the Fixed-Bed Reactor	543
19.1.4	Productivity of a Fixed-Bed Enzyme Reactor	547

18	Artificial Enzymes	511
18.1	Catalytic Antibodies	512
18.1.1	Principle of Catalytic Antibodies: Connection between Chemistry and Immunology	512
18.1.2	Test Reaction Selection, Haptens, Mechanisms, Stabilization	514
18.1.2.1	Mechanism of Antibody-Catalyzed Reactions	516
18.1.2.2	Stabilization of Charged Transition States	517
18.1.2.3	Effect of Antibodies as Entropy Traps	517
18.1.3	Breadth of Reactions Catalyzed by Antibodies	518
18.1.3.1	Fastest Antibody-Catalyzed Reaction in Comparison with Enzymes	518
18.1.3.2	Antibody-Catalyzed Reactions without Corresponding Enzyme Equivalent	518
18.1.3.3	Example of a Pericyclic Reaction: Claisen Rearrangement	518
18.1.3.4	Antibody Catalysts with Dual Activities	518
18.1.3.5	Scale-Up of an Antibody-Catalyzed Reaction	520
18.1.3.6	Perspective for Catalytic Antibodies	520
18.2	Other Proteinaceous Catalysts: Ribozymes and Enzyme Mimics	521
18.2.1	Ribozymes: RNA World before Protein World?	521
18.2.2	Proteinaceous Enzyme Mimics	521
18.3	Design of Novel Enzyme Activity: Enzyme Models (Synzymes)	523
18.3.1	Introduction	523
18.3.2	Enzyme Models on the Basis of the Binding Step: Diels–Alder Reaction	523
18.3.3	Enzyme Models with Binding and Catalytic Effects	525
18.4	Heterogenized/Immobilized Chiral Chemical Catalysts	526
18.4.1	Overview of Different Approaches	526
18.4.2	Immobilization with Polyamino Acids as Chiral Polymer Catalysts	526
18.4.3	Immobilization on Resins or other Insoluble Carriers	527
18.4.4	Heterogenization with Dendrimers	528
18.4.5	Retention of Heterogenized Chiral Chemical Catalysts in a Membrane Reactor	529
18.4.6	Recovery of Organometallic Catalysts by Phase Change: Liquid–Liquid Extraction	531
18.5	Tandem Enzyme Organometallic Catalysts	532
19	Design of Biocatalytic Processes	539
19.1	Design of Enzyme Processes: High-Fructose Corn Syrup (HFCS)	540
19.1.1	Manufacture of HFCS from Glucose with Glucose Isomerase (GI): Process Details	540
19.1.2	Mathematical Model for the Description of the Enzyme Kinetics of Glucose Isomerase (GI)	541
19.1.3	Evaluation of the Model of the GI Reaction in the Fixed-Bed Reactor	543
19.1.4	Productivity of a Fixed-Bed Enzyme Reactor	547

19.2	Processing of Fine Chemicals or Pharmaceutical Intermediates in an Enzyme Membrane Reactor	549
19.2.1	Introduction	549
19.2.2	Determination of Process Parameters of a Membrane Reactor	550
19.2.2.1	Case 1: Leakage through Membrane, no Deactivation	551
19.2.2.2	Case 2: Leakage through the Membrane and Deactivation of Enzyme	552
19.2.2.3	Design Criterion for EMRs	552
19.2.3	Large-Scale Applications of Membrane Reactors	553
19.2.3.1	Enantiomerically Pure l-Amino Acids for Infusion Solutions and as Building Blocks for New Drugs	553
19.2.3.2	Aqueous–Organic Membrane Reactors	554
19.2.3.3	Other Processes in Enzyme Membrane Reactors	554
19.3	Production of Enantiomerically Pure Hydrophobic Alcohols: Comparison of Different Process Routes and Reactor Configurations	556
19.3.1	Isolated Enzyme Approach	556
19.3.2	Whole-Cell Approach	559
19.3.3	Organometallic Catalyst Approach	561
19.3.4	Comparison of Different Catalytic Reduction Strategies	563
20	Comparison of Biological and Chemical Catalysts for Novel Processes	569
20.1	Criteria for the Judgment of (Bio-)catalytic Processes	570
20.1.1	Discussion: Jacobsen's Five Criteria	570
20.1.2	Comment on Jacobsen's Five Criteria	572
20.2	Position of Biocatalysis in Comparison to Chemical Catalysts for Novel Processes	575
20.2.1	Conditions and Framework for Processes of the Future	575
20.2.2	Ibuprofen (Painkiller)	577
20.2.3	Indigo (Blue Dye)	578
20.2.4	Menthol (Peppermint Flavoring Agent)	580
20.2.4.1	Separation of Diastereomeric Salt Pairs	580
20.2.4.2	Homogeneous Catalysis with Rh-BINAP	580
20.2.4.3	Lipase-Catalyzed Resolution of Racemic Menthol Esters	582
20.2.5	Ascorbic Acid (Vitamin C)	583
20.2.5.1	The Traditional Reichstein–Grüssner Synthesis	584
20.2.5.2	Two-Step Fermentation Process to 2-Ketogulonic Acid with Chemical Step to Ascorbic Acid	584
20.2.5.3	One-Step Fermentation to 2-Ketogulonic Acid with Chemical Step to Ascorbic Acid	585
20.3	Pathway Engineering through Metabolic Engineering	586
20.3.1	Pathway Engineering for Basic Chemicals: 1,3-Propanediol	586
20.3.2	Pathway Engineering for Pharmaceutical Intermediates: cis-Aminoindanol	588