

Contents

Preface XV

List of Contributors XVII

1	Fluorescence Assays for Biotransformations	1
	<i>Jean-Louis Reymond</i>	
1.1	Introduction	1
1.2	Alcohol Dehydrogenases (ADHs) and Aldolases	2
1.2.1	Chiral Fluorogenic ADH Substrates	2
1.2.2	Fluorogenic Aldolase Probes	3
1.2.3	Transaldolases and Transketolases	4
1.2.4	Enolase Probe	4
1.3	Lipases and Esterases	5
1.3.1	Assays on Solid Support	6
1.3.2	The Clips-O Substrates with Periodate	8
1.3.3	Esters of Fluorogenic Cyanohydrins and Hydroxyketones	9
1.3.4	Fluorogenic Acyloxymethyl Ethers	10
1.3.5	FRET-Lipase Probes	11
1.4	Other Hydrolases	11
1.4.1	Epoxide Hydrolases	11
1.4.2	Amidases and Proteases	13
1.4.3	Phosphatases	14
1.5	Baeyer-Villigerases	15
1.6	Conclusion	15
	Acknowledgment	16
	References	16
2	Immobilization as a Tool for Improving Enzymes	21
	<i>Ulf Hanefeld</i>	
2.1	Introduction	21
2.2	Adsorption/Electrostatic Interactions	22
2.2.1	Van der Waals Interactions	22
2.2.2	Hydrogen Bonds	25

2.2.3	Ionic Interactions	27
2.3	Encapsulation	31
2.4	Covalent Binding/Cross-linking	33
2.5	Conclusion	38
	Acknowledgments	38
	References	39
3	Continuous-flow Microchannel Reactors with Surface-immobilized Biocatalysts	43
	<i>Malene S. Thomsen and Bernd Nidetzky</i>	
3.1	Introduction	43
3.2	Biocatalytic Synthesis Using Microreaction Technology with Free and Immobilized Enzymes	44
3.3	Novel Microfluidic Immobilized Enzyme Reactors	45
3.3.1	Microreactor Design	45
3.3.2	Enzyme Immobilization	47
3.4	Enzymatic Hydrolysis of Lactose	48
3.4.1	Catalytic Effectiveness of Immobilized CelB	48
3.4.2	Continuous Conversion of Lactose	48
3.5	Biocatalytic Process Intensification Using Microreaction Technology	50
3.6	Conclusions and Outlook	51
	Acknowledgements	52
	References	52
4	Activity and Stability of Proteases in Hydrophilic Solvents	55
	<i>Lars Haastrup Pedersen, Sinthuwat Ritthitham, and Morten Kristensen</i>	
4.1	Introduction	55
4.2	Activity and Selectivity of Proteases in Synthesis of Carbohydrate Fatty Acid Esters	56
4.3	Enzyme Stability and Conformation	59
4.4	Solvent Engineering	63
4.5	Conclusion	64
	References	65
5	Importance of Enzyme Formulation for the Activity and Enantioselectivity of Lipases in Organic Solvents	67
	<i>Francesco Secundo</i>	
5.1	Introduction	67
5.2	Lipase Formulations and their Activity and Enantioselectivity in Neat Organic Solvent	68
5.3	Why do Additives Affect the Activity and Enantioselectivity of Lipases in Organic Solvent?	73
5.4	Conclusions	76
	References	76

6	Direct Esterification with Dry Mycelia of Molds: a (Stereo)selective, Mild and Efficient Method for Obtaining Structurally Diverse Esters	79
	<i>Francesco Molinari, Diego Romano, Raffaella Gandolfi, Lucia Gardossi, Ulf Hanefeld, Attilio Converti and Patrizia Spizzo</i>	
6.1	Mycelia and Biotransformations in Organic Media	79
6.2	Screening and Microbiological Aspects	79
6.3	Production of Acetate	81
6.4	Stereoselective Esterifications of Racemic Alcohols	83
6.5	Stereoselective Esterifications of Racemic Carboxylic Acids	85
6.6	Partition Phenomena and Equilibrium of Esterification Reactions	88
6.7	Conclusions	91
	References	91
7	Factors Affecting Enantioselectivity: Allosteric Effects	93
	<i>Elisabeth Egholm Jacobsen and Thorleif Anthonsen</i>	
7.1	How to Provide Enantiopure Compounds	93
7.1.1	Kinetic Resolution of Racemic Mixtures Catalyzed by Enzymes	94
7.1.2	Absolute Configurations in Resolution	95
7.2	Factors Affecting the Enantiomeric Ratio <i>E</i>	96
7.2.1	Is the <i>E</i> -value Really Constant?	96
7.2.2	Influence of the Reaction Medium on the <i>E</i> -value	96
7.2.3	Influence of Enzyme Immobilization on the <i>E</i> -value	97
7.2.4	Enzyme Inhibition	97
7.2.5	Enantioselective Inhibition and Activation: Allosteric Effects	97
7.2.6	The <i>E</i> -value of CALB is Influenced by R-Alcohols	99
7.2.7	Is a Changing <i>E</i> Caused by the Slow or the Fast Enantiomer?	102
7.3	Asymmetrization of Prochiral Compounds	103
7.3.1	Asymmetrization of Prochiral Dicarboxylates: Single-Step Process	103
7.3.2	Asymmetrization of Prochiral Diol: Double-Step Process	105
7.3.3	Is the e.e. Constant During Asymmetrization Reactions?	105
7.4	Conclusions	106
	References	107
8	Kinetic Resolution of <i>Sec</i>-alcohol in Non-conventional Media	109
	<i>Maja Habulin, Mateja Primožič and Željko Knez</i>	
8.1	Introduction	109
8.2	SCFs—Replacement for Organic Solvents in Biocatalysis	111
8.3	Effect of Pressure	112
8.4	Effect of the Acyl Donor/Alcohol Molar Ratio	114
8.5	ILs—Solvents for Sustainable Technology in Biocatalysis	114
8.6	ILs, Based on the N, N'-Dialkylimidazolium Cations as Reaction Media	116
8.7	ILs/SCFs Biphasic Systems as Promising Media for Biocatalysis	117

8.8	The [bmim][PF ₆]/SC-CO ₂ System as a Reaction Medium	117
8.9	Effect of Acyl Donor Concentration	119
8.10	Conclusion	120
	References	120
9	Strategies for the Biocatalytic Lipophilization of Phenolic Antioxidants	123
	<i>Maria H. Katsoura, Eleni Theodosiou, Haralambos Stamatis and Fragiskos N. Kolisis</i>	
9.1	Introduction	123
9.2	Materials and Methods	125
9.2.1	Materials	125
9.2.2	Enzymatic Acylation Procedure	125
9.2.3	Analytical Methods	125
9.2.4	Purification and Chemical Structure Determination of Esters	125
9.3	Results and Discussion	125
9.3.1	Modification of Natural Antioxidants in Organic Solvents	126
9.3.1.1	Enzymatic Acylation of Rutin and Silybin with Dicarboxylic Acids	126
9.3.1.2	Effect of Organic Solvent	127
9.3.1.3	Effect of Substrate Concentration	127
9.3.2	Modification of Natural Antioxidants in Ionic Liquid Media	128
9.3.2.1	Enzymatic Acylation of Natural Polyhydroxylated Compounds	128
9.3.2.2	Effect of Substrate Concentration	130
9.3.2.3	Effect of Acyl Donor Nature: Synthesis of Hybrid Antioxidants	130
9.4	Conclusions	131
	References	132
10	Biocatalysis Applied to the Synthesis of Nucleoside Analogs	135
	<i>Vicente Gotor</i>	
10.1	Introduction	135
10.2	Chemoenzymatic Modification of the Sugar	136
10.3	Resolution and Anomeric Separation	143
10.4	Biotransformations that Modify the Base	145
10.5	Transglycosylation for the Synthesis of Nucleosides	147
10.6	Summary	149
	References	150
11	Efficient Fructooligosaccharide Synthesis with a Fructosyltransferase from <i>Aspergillus aculeatus</i>	153
	<i>Francisco J. Plou, Miguel Alcalde, Iraj Ghazi, Lucía Fernández-Arrojo and Antonio Ballesteros</i>	
11.1	Introduction	153
11.2	Purification of Fructosyltransferase in Pectinex Ultra SP-L	155
11.3	Properties of Fructosyltransferase from <i>A. aculeatus</i>	157

11.3.1	Substrate Specificity	157
11.3.2	Effect of pH and Temperature	158
11.3.3	Influence of Chemicals	158
11.3.4	Kinetic Behavior	159
11.3.5	Fructooligosaccharide Production	159
11.4	Immobilization of Fructosyltransferase from <i>A. aculeatus</i>	161
11.4.1	Sepabeads EC-EP as Immobilization Carriers	161
11.4.2	Effect of pH and Ionic Strength on Immobilization	162
11.4.3	Application of Immobilized Biocatalysts to Fructooligosaccharide Synthesis	164
11.5	Fructooligosaccharide Production Using Sugar Beet Syrup and Molasses	164
11.5.1	Sugar Beet Syrup and Molasses as Low-cost Feedstock for Fructooligosaccharide Synthesis	164
11.5.2	Batch Production of Fructooligosaccharide	167
11.6	Conclusions	168
	Acknowledgments	168
	References	168
12	Hydantoin Racemase: the Key Enzyme for the Production of Optically Pure α-Amino Acids	173
	<i>Francisco Javier Las Heras-Vázquez, Josefa María Clemente-Jiménez, Sergio Martínez-Rodríguez and Felipe Rodríguez-Vico</i>	
12.1	Introduction	173
12.2	Search for New Hydantoin Racemases and Molecular Characterization	175
12.3	Biochemical Characterization of Hydantoin Racemase Enzymes	180
12.4	Substrate Enantioselectivity and Kinetic Analysis of Hydantoin Racemases	181
12.5	Proposal for a Reaction Mechanism of Hydantoin Racemase Enzymes	183
12.6	Design of a Tailormade Recombinant Biocatalyst Including Hydantoin Racemase Enzymes for Optically Pure D-Amino Acid Production	187
	Acknowledgments	192
	References	192
13	Chemo-enzymatic Deracemization Methods	195
	<i>Davide Tessaro, Gianluca Molla, Loredano Pollegioni and Stefano Servi</i>	
13.1	Introduction	195
13.2	Deracemization Methods for α - and β -Hydroxy Acids	196
13.2.1	Deracemization of Hydroxy Acids by DKR (Hydrolytic Enzymes + Ruthenium-based Racemization Catalysts)	197
13.2.2	Deracemization of Hydroxy Acids by DKR with a Two-enzyme System	198
13.2.3	Deracemization of Hydroxy Acids by Stereoinversion	199

13.2.4	Deracemization of Hydroxy Acids by Microbial Stereoinversion	200
13.3	Deracemization of α -Hydroxy Nitriles	201
13.4	Deracemization of α -Amino Acids	202
13.4.1	Deracemization of α -Amino Acids by Stereoinversion	202
13.4.1.1	Deracemization by Stereoinversion via the Two-enzyme System D-Amino Acid Oxidase and L-Amino Transferase	202
13.4.1.2	Deracemization by Stereoinversion via the Two-enzyme System D-Amino Acid Oxidase and L-Leucine Dehydrogenase	204
13.4.1.3	Deracemization by Stereoinversion via the Three-enzyme System L-Amino Acid Oxidase, D-Amino Transferase and Amino Acid Racemase	204
13.4.2	Deracemization of α -Amino Acids via DKR	205
13.4.2.1	Deracemization of α -Amino Acids via Enzyme-catalyzed DKR Coupled with <i>In Situ</i> Racemization	205
13.5	Useful Enzymes for Deracemization Methods	213
13.5.1	Amino Acid Oxidases	213
13.5.1.1	D-Amino Acid Oxidase (EC 1.4.3.3)	213
13.5.1.2	L-Amino Acid Oxidase (EC 1.4.3.2)	216
13.5.2	Amino Acid Racemases	217
13.5.2.1	PLP-dependent Racemases	217
13.5.2.2	PLP-independent Racemases	220
13.5.2.3	Mandelate Racemase (EC 5.1.2.2)	221
13.5.3	Transaminases	221
13.5.3.1	L-Amino Transferases (EC 2.6.1.x)	222
13.5.3.2	D-Amino Transferases (EC 2.6.1.21)	223
13.6	Summary and Outlook	223
	References	223
14	Nitrilases from Filamentous Fungi	229
	<i>Ludmila Martínková, Vojtech Vejvoda, Ondrej Kaplan, Vladimír Kren, Karel Bezouška and Maria Cantarella</i>	
14.1	Introduction	229
14.2	Distribution and Evolutionary Relationship of Fungal Nitrilases	230
14.2.1	Molecular Genetic Analysis	230
14.2.2	Selection and Screening of Nitrilase Activity	232
14.3	Structural Properties	234
14.4	Catalytic Properties	236
14.4.1	Reaction Mechanism	236
14.4.2	Substrate Specificity	238
14.4.3	Activity and Stability	240
14.5	Conclusions and Outlook	242
	Acknowledgment	243
	References	243

15	Nitrilase- and Nitrile Hydratase-catalyzed Enantioselective Preparation of Non-proteinogenic Amino Acids	247
	<i>Norbert Klempier and Margit Winkler</i>	
15.1	Introduction	247
15.2	Nitrile Hydratase/Amidase Biotransformations	249
15.2.1	Protecting Groups for Amino Nitriles	249
15.2.2	Enantioselective Hydrolysis of β -Amino Nitriles	250
15.3	Nitrilase Biotransformations	253
15.3.1	Enantioselective Hydrolysis of β -Amino Nitriles	253
15.3.2	Enantioselective Hydrolysis of γ -Amino Nitriles	255
15.3.3	Nitrile Hydratase Activity of Nitrilases	257
	References	258
16	Nitrilases in the Enantioselective Synthesis of α-Hydroxycarboxylic Acids	261
	<i>Fred van Rantwijk, Cesar Mateo, Andrzej Chmura, Bruno C. M. Fernandes and Roger A. Sheldon</i>	
16.1	Routes to Enantiomerically Pure α -Hydroxycarboxylic Acids	261
16.2	Nitrilase-mediated Hydrolysis of Cyanohydrins	262
16.3	A Biocatalytic Approach to Enantiopure 2-Hydroxycarboxylic Acids	264
16.4	Stabilization of NLases as Cross-linked Enzyme Aggregates	265
16.5	Hydrocyanation and Hydrolysis in a Biocatalytic Cascade	265
16.6	Nitrilases Acting as Nitrile Hydratases	267
16.7	Conclusion	270
	Acknowledgments	271
	References	271
17	UF-Membrane Bioreactors for Kinetics Characterization of Nitrile Hydratase–Amidase-catalyzed Reactions: a Short Survey	273
	<i>Maria Cantarella, Alberto Gallifuoco, Agata Spera, Laura Cantarella, Ondřej Kaplan and Ludmila Martínková</i>	
17.1	Introduction	273
17.2	Experiment Design	275
17.3	Temperature Dependence of the Nitrile Hydratase–Amidase Cascade System	275
17.4	CSMR Investigations	277
17.5	Substrate Concentration Effects on the Reaction Rate, Enzyme Stability, Substrate Conversion, and Reactor Capacity	279
17.6	Concluding Remarks	283
	Acknowledgments	285
	References	285

18	Enzymes Catalyzing C—C Bond Formation for the Synthesis of Monosaccharide Analogs	287
	<i>Laurence Hecquet, Virgil Hélaine, Franck Charmantray and Marielle Lemaire</i>	
18.1	Introduction	287
18.2	Recent Syntheses Involving Transketolase and Fructose-1,6-bisphosphate Aldolase	287
18.2.1	DHAP Syntheses	288
18.2.1.1	DHAP Synthesis from Dihydroxyacetone	289
18.2.1.2	DHAP Synthesis from Rac-Glycidol	290
18.2.2	Synthesis of Aminocyclitols	291
18.2.3	Synthesis of 5-D-Xylulose and 5-D-Xylose Analogs	293
18.2.3.1	Synthesis of 5-halo-D-xylulose	293
18.2.3.2	Synthesis of 5-thio-D-xylopyranose	21 293
18.3	Modification of Substrate Specificity of Yeast Transketolase	295
18.4	Conclusion	296
	References	297
19	Novel Strategies in Aldolase-catalyzed Synthesis of Iminosugars	299
	<i>Pere Clapés, Georg A. Sprenger and Jesús Joglar</i>	
19.1	Introduction	299
19.2	DHAP-Aldolase-mediated Synthesis of Iminosugars from N-Cbz-amino Aldehydes	301
19.2.1	Reaction Media	301
19.2.2	Aldolase-catalyzed Aldol Additions of DHAP to N-Cbz-Amino Aldehydes	301
19.2.3	Effect of N-Protecting Groups	304
19.2.4	Synthesis of Iminosugars: Reductive Amination	306
19.3	D-Fructose-6-Phosphate Aldolase as Catalyst for Iminosugar Synthesis	307
19.4	Summary and Outlook	309
	References	309
20	Biocatalytic Asymmetric Oxidations with Oxygen	313
	<i>Roland Wohlgemuth</i>	
20.1	Introduction	313
20.2	Biocatalytic Asymmetric Oxidations with Oxidases	317
20.3	Biocatalytic Asymmetric Oxidations with Peroxidases	319
20.4	Biocatalytic Asymmetric Oxidations with Dehydrogenases	320
20.5	Biocatalytic Asymmetric Oxidations with Monooxygenases	321
20.6	Biocatalytic Asymmetric Oxidations with Dioxygenases	325
20.7	Biocatalytic Asymmetric Oxidations with Other Enzymes	328
20.8	Outlook	331
	Acknowledgments	331
	References	332

21	Second Generation Baeyer–Villiger Biocatalysts	339
	<i>Veronique Alphand, Marco W. Fraaije, Marko D. Mihovilovic and Gianluca Ottolina</i>	
21.1	Introduction	339
21.2	BVMO Enzyme Platform	341
21.3	Engineering of BVMOs	342
21.4	Baeyer–Villiger Biooxidation in Synthetic Chemistry	347
21.4.1	Chemoselectivity	347
21.4.2	Dynamic Kinetic Resolutions	350
21.4.3	Regio- and Stereoselectivity	351
21.4.4	Natural Product and Bioactive Compound Synthesis	354
21.5	BVMOs in Stereoselective Sulfoxidations	357
21.6	Towards a Technology Platform	358
21.6.1	Fermentation Up-Scaling	358
21.6.1.1	Whole Cells	358
21.6.1.2	Enzyme	361
21.6.2	Immobilization of BVMOs	361
21.6.3	Self-sufficient Fusion Protein BVMOs	361
21.7	Outlook	363
	References	363
	Index	369