

Contents

Preface *xiii*

Part I Overview of Industrial Enzyme Applications and Key Technologies 1

- 1.1 Industrial Enzyme Applications – Overview and Historic Perspective 3**
Oliver May
 - 1.1.1 Prehistoric Applications 3
 - 1.1.2 Growing the Scientific Basis 5
 - 1.1.3 The Beginning of Industrial Applications and the Emerging Enzyme Industry 12
 - References 21
- 1.2 Enzyme Development Technologies 25**
Andreas Vogel
 - 1.2.1 Introduction 25
 - 1.2.2 Identification of Wild-Type Enzymes 26
 - 1.2.2.1 Selection Parameters for Starting Enzymes 28
 - 1.2.3 Enzyme Engineering 30
 - 1.2.3.1 Types of Enzyme Modifications 30
 - 1.2.3.2 General Engineering Strategies. Library Design and Generation 30
 - 1.2.3.3 Screening for Better Enzymes 37
 - 1.2.4 Impact of Enzyme Development Technologies Today and Tomorrow 38
 - Acknowledgments 41
 - References 41
- 1.3 Eukaryotic Expression Systems for Industrial Enzymes 47**
Lukas Rieder, Nico Teuschler, Katharina Ebner, and Anton Glieder
 - 1.3.1 Eukaryotic Enzyme Production Systems 47
 - 1.3.2 Special Considerations for Working with Eukaryotic Expression Systems 47

1.3.2.1	Choice of Expression Host	47
1.3.2.2	Comparison of Cell Structure and Their Influence on Molecular Biology	49
1.3.3	Differences in Vector Design for Eukaryotic and Prokaryotic Hosts	51
1.3.4	Differences in Regulation of Gene Expression in Eukaryotes and Prokaryotes	56
1.3.4.1	Different Types of Promoters	58
1.3.5	Industrial Enzyme Production	58
1.3.6	Enzyme Production on Industrial Scale	61
1.3.6.1	Homologous Protein Production	61
1.3.6.2	Heterologous Protein Production	62
	References	63

1.4 Process Considerations for the Application of Enzymes 71

Selin Kara and Andreas Liese

1.4.1	Biocatalyst Types Used in Industrial Processes	71
1.4.2	Enzyme Immobilization for Biocatalytic Processes	74
1.4.3	Reaction Medium Applied in Enzymatic Catalysis	76
1.4.3.1	Monophasic Systems – Organic Media	77
1.4.3.2	Multiphasic Systems – Liquid/Liquid Mixtures	80
1.4.3.3	Multiphasic Systems – Gas/Liquid Mixtures	83
1.4.3.4	Multiphasic Systems – Solid/Liquid Mixtures	84
1.4.4	Appropriate Reactor Types in Enzyme Catalysis	87
1.4.5	Assessment Criteria for Enzymatic Applications	90
	References	92

Part II Enzyme Applications for the Food Industry 95

2.1 Enzymes Used in Baking 97

Joke A. Putseys and Margot E.F. Schooneveld-Bergmans

2.1.1	Introduction	97
2.1.2	The Baking Process – The Baker's Needs	98
2.1.2.1	Flour Quality and Standardization	98
2.1.2.2	Mixing and Dough Handling	100
2.1.2.3	Fermentation and Dough Stability	105
2.1.2.4	Baking and Oven Spring	109
2.1.3	The Bread Quality – The Consumers' Needs	111
2.1.3.1	Color and Flavor	111
2.1.3.2	Shelf Life	112
2.1.4	Trends and Opportunities for Baking Enzymes	116
2.1.4.1	Fine Baking and Confectionary	116
2.1.4.2	Consumer Preference: Health, Individual Values, and Convenience	117
2.1.5	Conclusion	118
	References	119

2.2	Protein Modification to Meet the Demands of the Food Industry	125
	<i>Andrew Ellis</i>	
2.2.1	Food Proteins	125
2.2.2	Processing of Food Protein	127
2.2.3	Enzymes in the Processing of Food Proteins	127
2.2.4	Food Protein Value Chain	130
2.2.5	Recent Enzyme Developments	131
2.2.5.1	Simple Protein Modification (Value Level 3)	131
2.2.5.1.1	Developing Microbial Alternatives to Plant and Animal Enzymes	131
2.2.5.2	Specialized Enzyme Modification (Value Level 4)	134
2.2.5.2.1	Whey Protein Hydrolysates	134
2.2.5.2.2	Plant Protein Hydrolysates	134
2.2.5.3	Highly Specific Protein Modification (Value Level 5)	135
2.2.5.3.1	Gluten Modification	135
2.2.5.3.2	Acrylamide Reduction	135
2.2.5.3.3	Bioactive Peptides	136
2.2.6	Enzymes to Meet Future Needs	137
	Acknowledgments	139
	References	139
2.3	Dairy Enzymes	143
	<i>Peter Dekker</i>	
2.3.1	Introduction	143
2.3.2	Coagulants	145
2.3.2.1	Traditional Rennets	147
2.3.2.2	Microbial Rennets	148
2.3.2.3	Fermentation Produced Chymosin	151
2.3.3	Ripening Enzymes	152
2.3.3.1	Proteases/Peptidases	153
2.3.3.2	Lipases/Esterases	154
2.3.4	Lactases	154
2.3.4.1	Neutral Lactase	156
2.3.4.2	Acid Lactase	158
2.3.4.3	GOS Production	158
2.3.5	Miscellaneous Enzymes	161
2.3.5.1	Oxidases/Peroxidases	161
2.3.5.2	Phospholipases	162
2.3.5.3	Cross-linking Enzymes	162
2.3.5.4	Preservation	163
2.3.6	New Developments	163
	References	163

2.4	Enzymatic Process for the Synthesis of Cellobiose	167
	<i>Birgit Brucher and Thomas Häßler</i>	
2.4.1	Enzymatic Synthesis of Cellobiose	167
2.4.2	Cellobiose – Properties and Applications	168
2.4.3	Existing Routes for Cellobiose Synthesis	170
2.4.4	Enzyme Development	171
2.4.5	Process Development	173
2.4.5.1	Synthesis of Cellobiose	174
2.4.5.2	Purification of Cellobiose	174
2.4.6	Summary and Future Perspective	176
	References	176
2.5	Emerging Field – Synthesis of Complex Carbohydrates. Case Study on HMOs	179
	<i>Dora Molnar-Gabor, Markus J. Hederos, Sebastian Bartsch, and Andreas Vogel</i>	
2.5.1	Introduction to Human Milk Oligosaccharides (HMOs)	179
2.5.1.1	Discovery and Function of HMOs	179
2.5.1.2	Structure of HMOs	180
2.5.1.3	HMO Production, Regulatory Authorizations, and Commercial Launch – Historical Overview	181
2.5.2	Glycom A/S Technologies Toward Commercial HMO Production	184
2.5.2.1	Whole Cell Microbial Fermentation to HMOs (<i>In Vivo</i> Process)	185
2.5.2.2	The Glycom <i>In Vitro</i> Concept to Diversify HMO Blends	187
2.5.2.3	Validation of the HMO Diversification Concept with Non-optimized Enzymes	187
2.5.3	Enzyme Development	189
2.5.3.1	Optimization of the α 1-3/4 Transfucosidase	189
2.5.3.2	Optimization of the α 2-6 Transsialidase	192
2.5.4	Applications of the Optimized Enzymes for the HMO Profiles	195
2.5.4.1	Scale-Up of the Lacto- <i>N</i> -fucopentaose III (LNFP-III), Sialyl Lacto- <i>N</i> -neotetraose (LST-c), and Sialyl Lacto- <i>N</i> -tetraose (LST-a) HMO Profiles	195
2.5.5	Conclusion and Perspective	197
	References	198

Part III Enzyme Applications for Human and Animal Nutrition 203

3.1	Enzymes for Human Nutrition and Health	205
	<i>Yoshihiko Hirose</i>	
3.1.1	Introduction	205
3.1.2	Current Problems of Enzymes in Healthcare Business	205
3.1.3	Enzymes in Existing Healthcare Products	206
3.1.3.1	Digestive Enzymes	206

3.1.3.1.1	Digestive Enzymes in United States	206
3.1.3.1.2	Therapeutic Digestive Enzymes	207
3.1.3.2	Acid Lactase	207
3.1.3.3	α -Galactosidase (ADG)	208
3.1.3.4	Dextranase	208
3.1.3.5	Glucose Oxidase	208
3.1.3.6	Acetobacter Enzymes	210
3.1.3.7	Laccase (Polyphenol Oxidase)	210
3.1.4	New Enzyme Developments in Healthcare Products	211
3.1.4.1	Transglucosidase	211
3.1.4.2	Laccase	211
	References	215
3.2	Enzyme Technology for Detoxification of Mycotoxins in Animal Feed	219
	<i>Dieter Moll</i>	
3.2.1	Introduction to Mycotoxins	219
3.2.2	Mycotoxin Mitigation Strategies	220
3.2.3	Enzyme Applications	224
3.2.4	FUMzyme®	225
3.2.4.1	The Substrate: Fumonisin	225
3.2.4.2	Enzyme Discovery	227
3.2.4.3	Enzyme Selection	230
3.2.4.4	Enzyme Activity Assays	232
3.2.4.5	Enzyme Characterization and Evaluation	233
3.2.4.6	Enzyme Feeding Trials and Biomarker Analysis	234
3.2.4.7	Enzyme Engineering	237
3.2.4.8	Enzyme Production	238
3.2.4.9	Enzyme Registration	239
3.2.5	Future Mycotoxinases	240
3.2.6	Conclusions	242
	References	243
3.3	Phytases for Feed Applications	255
	<i>Nikolay Outchkourov and Spas Petkov</i>	
3.3.1	Phytase As a Feed Enzyme: Introduction and Significance	255
3.3.2	Historical Overview of the Phytase Market Development	256
3.3.3	From Phytate to Phosphorus: Step by Step Action of the Phytase	259
3.3.3.1	Properties of Phytate	259
3.3.3.2	Phytases Structural and Functional Classification	260
3.3.3.2.1	Phytases from the Histidine Acid Phosphatases (HAP) Superfamily	261
3.3.3.2.2	β -Propeller Phytase (BPP)	261
3.3.3.2.3	Cysteine Phytase (CPhy)	263
3.3.3.2.4	Purple Acid Phytases (PAPhy)	263
3.3.3.2.5	Classification of the Phytases Based on Phytate Dephosphorylation Steps	263

3.3.4	Nutritional Values of Phytase in Animal Feed	265
3.3.5	Phytase Application As Feed Additive	265
3.3.6	Effective Phytate Hydrolysis in the Upper Digestive Tract of the Animal	266
3.3.7	Kinetic Description of Ideal Phytases	269
3.3.8	Resistance to Low pH and Proteases	271
3.3.9	Temperature Stability	271
3.3.10	In lieu of Conclusion: Lessons from Phytase Super Dosing Trials	274
	References	275

Part IV Enzymes for Biorefinery Applications 287

4.1	Enzymes for Pulp and Paper Applications	289
	<i>Debayan Ghosh, Bikas Saha, and Baljeet Singh</i>	
4.1.1	Refining and Fiber Development Enzyme	290
4.1.1.1	Microscopic Evaluation	291
4.1.1.2	Evaluation of Enzyme-Treated Handsheets	293
4.1.1.2.1	Case Study 1	293
4.1.1.2.2	Case Study 2	295
4.1.2	Drainage Improvement Enzyme	296
4.1.2.1	Case Study 3	299
4.1.2.2	Case Study 4	300
4.1.3	Stickies Control Enzyme	301
4.1.3.1	Case Study 5	303
4.1.4	Deinking Enzymes	306
4.1.4.1	Case Study 6	307
4.1.5	Hardwood Vessel Breaking Enzyme	308
4.1.5.1	Fiber Tester Image Analysis	308
4.1.6	Native Starch Conversion Enzyme	310
4.1.7	Bleach Boosting Enzyme	312
4.1.7.1	Common Bleaching Agents	312
4.1.7.1.1	Case Study 7	313
4.1.7.2	Overcoming Challenges Faced by Bleaching Enzymes in Pulp and Paper industry	315
4.1.8	Paper Mill Effluent Treatment Enzymes	315
4.1.8.1	Case Study 8	316
4.1.9	Slushing Enzyme	317
4.1.9.1	Case Study 9	317
4.1.9.2	Role of Enzymes in Pulp and Paper Industry – End Note!	318
	References	319
4.2	Enzymes in Vegetable Oil Degumming Processes	323
	<i>Arjen Sein, Tim Hitchman, and Chris L.G. Dayton</i>	
4.2.1	Introduction	323
4.2.2	General Seed Oil Processes	324
4.2.2.1	Phospholipids	325

4.2.2.2	A Molecular View of the Degumming Process	327
4.2.3	Enzymatic Degumming	330
4.2.3.1	Phospholipase C	331
4.2.3.2	Ways to Cope with Poor Conversion/Poor Quality Oils in PLC-Based Processes	333
4.2.3.3	Phospholipase A	336
4.2.4	Enzymatic Degumming in Industrial Practice	337
4.2.4.1	Introduction Hurdles	341
4.2.5	Other Applications of Enzymes in Oil – Outlook	343
4.2.5.1	Enzymatic Interesterification of Triglyceride Oils	343
4.2.5.2	Biodiesel	344
4.2.5.3	Enzyme-Assisted Decoloring	344
4.2.5.4	Enzyme-Assisted Oil Extraction	344
4.2.6	Conclusion	345
	Acknowledgments	345
	References	345

Part V Enzymes used in Fine Chemical Production 351

5.1	KREDs: Toward Green, Cost-Effective, and Efficient Chiral Alcohol Generation	353
	<i>Chris Micklitsch, Da Duan, and Margie Borra-Garske</i>	
5.1.1	Introduction	353
5.1.2	Ketoreductases	355
5.1.3	Cofactor Recycling	356
5.1.4	CodeEvolver® Protein Engineering Technology	358
5.1.5	Reduction of a Wide Range of Ketones/Aldehydes	358
5.1.6	Critical Selectivity Tools for Enantiopure Asymmetric Carbonyl Reduction	364
5.1.7	Examples of Improved KREDs for Improved Manufacturing	369
5.1.8	KREDs: Going Green and Saving Green	373
	References	377
5.2	An Aldolase for the Synthesis of the Statin Side Chain	385
	<i>Martin Schürmann</i>	
5.2.1	Introduction – Biocatalysis	385
5.2.1.1	Enzymes as Biocatalysts in Chemical Process	385
5.2.1.2	Biocatalytic Routes to the Statin Side Chain	387
5.2.2	The Aldolase DERA in Application	387
5.2.2.1	DERA-Catalyzed Aldol Reactions	387
5.2.2.2	Feasibility Phase of DERA-Enabled Statin Side Chain Process	390
5.2.3	Directed Evolution and Protein Engineering to Improve DERA	392
5.2.3.1	Rational Design	392
5.2.3.2	Directed Evolution of DERA	394
5.2.3.3	Other Approaches to Suitable or Improved DERAs	396

5.2.3.4	Other Applications of Process Intermediates and the DERA Technology	397
5.2.4	Conclusions	398
	Acknowledgments	400
	References	401
	Index	405