

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

1.1.3.1 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

1.2.4.1 Acknowledgments 41

1.2.4.2 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 |
| | <i>Selin Kara and Andreas Liese</i> | |
| 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 |
| | <i>Joke A. Putseys and Margot E.F. Schooneveld-Bergmans</i> | |
| 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 | Phopholipases | 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

Dieter Moll

| | | |
|---------|--|-----|
| 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: Fumonisins | 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

Nikolay Ouchkourov and Spas Petkov

| | | |
|-----------|---|-----|
| 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 |
|--------------|-----|