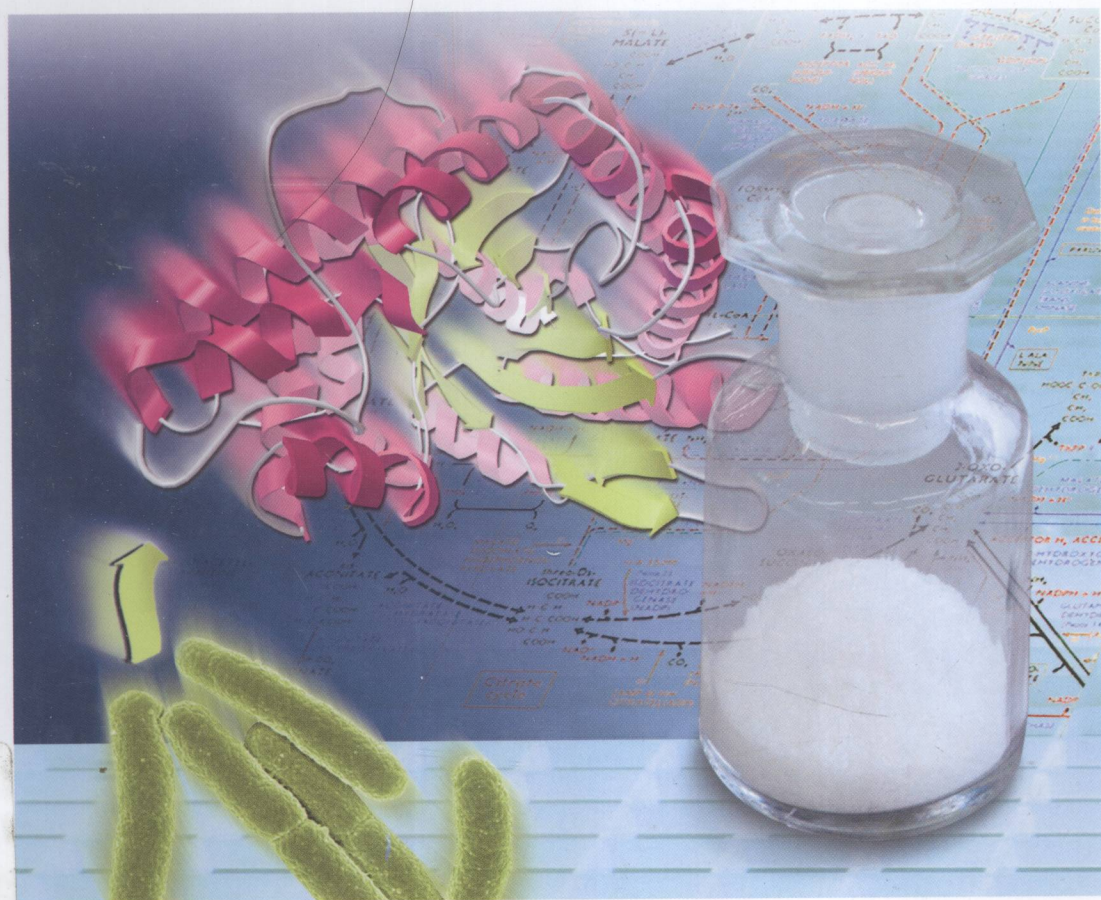


Edited by Wolf-Dieter Fessner
and Thorleif Anthonsen

 WILEY-VCH

Modern Biocatalysis

Stereoselective
and Environmentally Friendly Reactions



Q81U
M689

Modern Biocatalysis

Stereoselective and Environmentally Friendly Reactions

Edited by

Wolf-Dieter Fessner and Thorleif Anthonsen



WILEY-
VCH



E2009002829

WILEY-VCH Verlag GmbH & Co. KGaA

The Editors

Prof. Dr. Wolf-Dieter Fessner

TU Darmstadt, Inst. f. Organische Chemie und Biochemie
Petersenstr. 22
64287 Darmstadt

Prof. Dr. Thorleif Anthonsen

Norwegian Unvi. of Science & Technology, Dept. of Chemistry
Hogskoleringen 5
7491 Trondheim
Norwegen

Cover Illustration:

Background: Modified part of the "Biochemical Pathways Chart", 4th edition, by courtesy of F. Hoffmann-La Roche Ltd.

Protein structure taken from the RSCB Protein Data Base and modified:
PDB ID: 1ADO

N. Blom, J. Sygusch, Product binding and role of the C-terminal region in class I D-fructose 1,6-bisphosphate aldolase, *Nat. Struct. Biol.* **4** (1997), 36–39

■ All books published by Wiley-VCH are carefully produced. Nevertheless, authors, editors, and publisher do not warrant the information contained in these books, including this book, to be free of errors. Readers are advised to keep in mind that statements, data, illustrations, procedural details or other items may inadvertently be inaccurate.

Library of Congress Card No.:

applied for

British Library Cataloguing-in-Publication Data

A catalogue record for this book is available from the British Library.

Bibliographic information published by the Deutsche Nationalbibliothek

The Deutsche Nationalbibliothek lists this publication in the Deutsche Nationalbibliografie; detailed bibliographic data are available on the Internet at <http://dnb.d-nb.de>.

© 2009 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim

All rights reserved (including those of translation into other languages). No part of this book may be reproduced in any form—by photoprinting, microfilm, or any other means—nor transmitted or translated into a machine language without written permission from the publishers.
Registered names, trademarks, etc. used in this book, even when not specifically marked as such, are not to be considered unprotected by law.

Typesetting SNP Best-set Typesetter Ltd., Hong Kong

Printing Strauss GmbH, Mörlenbach

Binding Litges & Dopf GmbH, Heppenheim

Printed in the Federal Republic of Germany
Printed on acid-free paper

ISBN: 978-3-527-32071-4

Modern Biocatalysis

Edited by

Wolf-Dieter Fessner and

Thorleif Anthonsen

Related Titles

Sheldon, R. A., Arends, I., Hanefeld, U

Green Chemistry and Catalysis

2007

Hardcover

ISBN: 978-3-527-30715-9

Aehle, W. (ed.)

Enzymes in Industry Production and Applications

2007

Hardcover

ISBN: 978-3-527-31689-2

Liese, A., Seelbach, K., Wandrey, C.
(eds.)

Industrial Biotransformations Second, Completely Revised and Extended Edition

2006

Hardcover

ISBN: 978-3-527-31001-2

Garcia-Junceda, E. (ed.)

Multi-Step Enzyme Catalysis

Biotransformations and Chemoenzymatic Synthesis

2008

Hardcover

ISBN: 978-3-527-31921-3

Gotor, V., Alfonso, I., García-Urdiales, E. (eds.)

Asymmetric Organic Synthesis with Enzymes

2008

Hardcover

ISBN: 978-3-527-31825-4

Rothenberg, G.

Catalysis

Concepts and Green Applications

2008

Hardcover

ISBN: 978-3-527-31824-7



Preface

Asymmetric compounds cover a steadily increasing market share, e.g. as fine chemicals, chiral intermediates or pharmaceutical ingredients. The worldwide commercial volume of single isomers of chiral drugs currently approaches US\$200 billion. Chirality dictates how stereoisomers interact with biological systems. Because of the often much improved biological specificity profiles of pure enantiomers as compared to their racemates, methods for resolving racemates and for preparing single enantiomers are in high demand. For the challenge of asymmetric synthesis to generate complex chiral compounds in high enantiomeric purity and yield, biocatalysis offers a tremendous advantage due to the homochiral nature of protein catalysts, which offer unparalleled levels of stereoselectivity and reaction specificity, in addition to their remarkable catalytic efficiency under mild conditions and regenerative production from biological materials. Breakthrough technologies in modern molecular biotechnology research, development and application have recently paved the way for the rapid discovery and engineering of novel enzymes, and today allow the generation of biocatalysts that are optimally adaptable to even demanding industrial process parameters. Such features form the fundamentals of so-called white biotechnology – the use of microorganisms and enzymes for industrial chemical production, which currently strongly benefits from political support because of its impact on sustainable development, lower energy consumption and independence from fossil raw materials. Rising oil prices from a shortage of crude oil resources and political uncertainty in oil-producing countries; global warming due to greenhouse gases; increasing population; and environmental pollution are strong drivers for this trend.

Utilization of biocatalysis for chiral chemical synthesis and pharmaceutical manufacturing has come a long way, from highly specific niche applications a century ago, through hundreds of small to medium scale industrial processes in the fine chemical sector, e.g. including antibiotics, to even bulk processes for non-chiral commodity chemicals such as acrylamide. The conversion of traditional industrial processes to biotechnological alternatives is still at an early stage, because high costs in the development and adaptation of new processes have slowed down a substitution; development cycles for such biocatalytic processes used to be longer than anticipated and considerably longer than those for comparable chemical process alternatives. In the long run, however, biocatalytic processes have often

proven to be economically feasible, ecologically advantageous and more sustainable than current chemical technologies because of the intrinsic advantages of biocatalysts in higher reaction selectivity, milder reaction conditions and potential use of inexpensive regenerable resources. After all, the tools of the trade have changed dramatically over the past decade, and with current advanced technology in protein design and engineering, further developments are expected to strongly gain momentum for the immediate future. As one of the most impressive examples, the newly developed one-step fermentation process for vitamin B₂ at BASF has cut CO₂ emissions by 30%, production costs by 40%, consumption of resources by 60% and waste generation by 95% when compared to the conventional eight-step chemical synthesis.

Still, the field of biocatalysis research for preparative synthesis poses a range of intellectual frontiers and needs further developments to broaden the range of applicable reaction types, addressable target structures and advanced method integration. There is both general and industrial interest in further research to extend the scope of biocatalysis for asymmetric synthesis, and a collaboration of organic chemists, biochemists, molecular geneticists and biotechnical engineers is needed for success. The development of biocatalytic methodologies undoubtedly requires strong interdisciplinary and transdisciplinary research cooperation, and meeting the challenges for an environmentally friendly, sustainable process design adds another dimension, from the supply and efficient use of raw materials to the minimization and recycling of enzymes, by-products and waste under economical constraints. This book summarizes the efforts and current state of the art in several important arenas of biocatalysis research that has been coordinated within the activities of the European Union-funded COST network D25, entitled 'Applied Biocatalysis: Stereoselective and Environmentally Friendly Reactions Catalysed by Enzymes', over the last five years. The topics of the chapters span from modern assay technologies for enzyme screening over different factors influencing enzyme selectivities, including the consequences from enzyme formulation and various solvent effects, to the manifold of preparative applications. More than half of the book chapters deal with the various conceptual strategies and synthetic opportunities available for the rational synthesis or targeted modification of different compound classes, such as phenolic natural products, nucleoside analogs, monosaccharides and oligosaccharides, iminosugars, proteinogenic and non-proteinogenic amino acids, nitriles, hydroxy acids, and various oxidation products, including lactones from enzymatic Baeyer–Villiger reactions.

We are grateful to all those friends and colleagues who helped to start, and then participated in, the D25 action and who kindly contributed to this project as authors of dedicated and informative chapters in order to share their expertise with you. It is our hope that this volume will encourage scientific discussion and foster imaginative new developments in applied biocatalysis for tomorrow's novel applications.

Wolf-Dieter Fessner and Thorleif Anthonson
Darmstadt and Trondheim, October 2008

List of Contributors

Miguel Alcalde

Departamento de Biocatálisis
Instituto de Catálisis y
Petroquímica
CSIC
Cantoblanco
28049 Madrid
Spain

Veronique Alphand

Université Paul Cézanne
Biosciences–FRE CNRS 3005
Case 432
Av. Escadrille Normandie-Niemen
13397 Marseille cedex 20
France

Thorleif Anthonen

Norwegian University of Science
and Technology
Department of Chemistry
7491 Trondheim
Norway

Antonio Ballesteros

Departamento de Biocatálisis
Instituto de Catálisis y
Petroquímica
CSIC
Cantoblanco
28049 Madrid
Spain

Karel Bezouška

Charles University Prague
Faculty of Science
Department of Biochemistry
128 40 Prague
Czech Republic

Laura Cantarella

Department of Industrial Engineering
University of Cassino
Via di Biasio 43
03043 Cassino (FR)
Italy

Maria Cantarella

University of L'Aquila
Department of Chemistry
Chemical Engineering and Materials
67040 Monteluco di Roio–L'Aquila
Italy

Franck Charmantray

Université Blaise Pascal
CNRS
UMR 6504–SEESIB
63177 Aubière Cedex
France

Andrzej Chmura

Delft University of Technology
Department of Biotechnology
Laboratory of Biocatalysis and
Organic Chemistry
Julianalaan 136
2628 BL Delft
The Netherlands

Pere Clapés

Catalonia Institute for Advanced
Chemistry (IQAC)-CSIC. Group
of Biotransformation and
Bioactive Molecules
Jordi Girona 18-26
08034 Barcelona
Spain

Josefa María Clemente-Jiménez

Departamento de Química-Física
Bioquímica y Química Inorgánica
Edificio CITE I
Carretera de Sacramento S/N
La Cañada de San Urbano
04120 Almería
Spain

Attilio Converti

University of Genoa
Department of Chemical and
Process Engineering 'G.B.
Bonino'
Via Opera Pia 15
16145 Genoa
Italy

Bruno C. M. Fernandes

Delft University of Technology
Department of Biotechnology
Laboratory of Biocatalysis and
Organic Chemistry
Julianalaan 136
2628 BL Delft
The Netherlands

Lucía Fernández-Arrojo

Departamento de Biocatálisis
Instituto de Catálisis y Petroleoquímica
CSIC
Cantoblanco
28049 Madrid
Spain

Marco W. Fraaije

University of Groningen
Groningen Biomolecular Sciences and
Biotechnology Institute
Biochemical Laboratory
Nijenborgh 4
9747 AG Groningen
The Netherlands

Alberto Gallifuoco

University of L'Aquila
Department of Chemistry
Chemical Engineering and Materials
67040 L'Aquila
Italy

Raffaella Gandolfi

University of Milan
Institute of Organic Chemistry
'Alessandro Marchesini'
Via Venezian 21
20133 Milan
Italy

Lucia Gardossi

Università degli Studi di Trieste
Dipartimento di Scienze Farmaceutiche
Laboratory of Applied and
Computational Biocatalysis
Piazzale Europa 1
34127 Trieste
Italia

Iraj Ghazi

Departamento de Biocatálisis
 Instituto de Catálisis y
 Petroleoquímica
 CSIC
 Cantoblanco
 28049 Madrid
 Spain

Vicente Gotor

Universidad de Oviedo
 Departamento de Química
 Orgánica e Inorgánica
 Instituto de Biotecnología de
 Asturias
 33006 Oviedo
 Spain

Maja Habulin

University of Maribor
 Faculty of Chemistry and
 Chemical Engineering
 Laboratory for Separation
 Processes and Product Design
 Smetanova 17
 2000 Maribor
 Slovenia

Ulf Haneveld

Delft University of Technology
 Department of Biotechnology
 Biocatalysis and Organic
 Chemistry
 Julianalaan 136
 2628 BL Delft
 The Netherlands

Laurence Hecquet

Université Blaise Pascal
 Synthèse et Etudes de Systèmes à
 Intérêt Biologique
 UMR 6504
 24 avenue des Landais
 63177 Aubière Cedex
 France

Virgil Hélaine

Université Blaise Pascal
 CNRS
 UMR 6504–SEESIB
 63177 Aubière Cedex
 France

Francisco Javier Las Heras-Vázquez

Departamento de Química-Física
 Bioquímica y Química Inorgánica
 Edificio CITE I
 Carretera de Sacramento S/N
 La Cañada de San Urbano
 04120 Almería
 Spain

Elisabeth Egholm Jacobsen

Norwegian University of Science and
 Technology
 Department of Chemistry
 7491 Trondheim
 Norway

Jesús Joglar

Catalonia Institute for Advanced
 Chemistry (IQAC)-CSIC.
 Group of Biotransformation
 and Bioactive Molecules
 Jordi Girona 18-26
 08034 Barcelona
 Spain

Ondrej Kaplan

Academy of Sciences of the Czech
 Republic
 Institute of Microbiology
 Center of Biocatalysis and
 Biotransformation
 142 20 Prague
 Czech Republic

Maria H. Katsoura

University of Ioannina
Department of Biological
Applications and Technologies
Laboratory of Biotechnology
45110 Ioannina
Greece

Norbert Klempier

Technische Universität Graz
Institut für Organische Chemie
Stremayrgasse 16
8010 Graz
Austria

Željko Knez

University of Maribor
Faculty of Chemistry and
Chemical Engineering
Laboratory for Separation
Processes and Product Design
Smetanova 17
2000 Maribor
Slovenia

Fragiskos N. Kolisis

National Technical University
of Athens
Chemical Engineering
Department
Biotechnology Laboratory
5 Iroon Polytechniou Str.
Zografou Campus
15700 Athens
Greece

Vladimír Kren

Academy of Sciences of the Czech
Republic
Institute of Microbiology
Center of Biocatalysis and
Biotransformation
142 20 Prague
Czech Republic

Morten Kristensen

Arinco Arla Foods Amba
Mælkevejen 4
6920 Videbæk
Denmark

Marielle Lemaire

Université Blaise Pascal
CNRS
UMR 6504–SEESIB
63177 Aubière Cedex
France

Sergio Martínez-Rodríguez

Departamento de Química-Física
Bioquímica y Química Inorgánica
Edificio CITE I
Carretera de Sacramento S/N
La Cañada de San Urbano
04120 Almería
Spain

Ludmila Martínková

Academy of Sciences of the Czech
Republic
Institute of Microbiology
Center of Biocatalysis and
Biotransformation
142 20 Prague
Czech Republic

Cesar Mateo

Delft University of Technology
Department of Biotechnology
Laboratory of Biocatalysis and Organic
Chemistry
Julianalaan 136
2628 BL Delft
The Netherlands

Marko D. Mihovilovic

Vienna University of Technology
 Institute for Applied Synthetic
 Chemistry
 Getreidemarkt 9
 1060 Vienna
 Austria

Francesco Molinari

University of Milan
 Department of Food Science and
 Microbiology
 Via Celoria 2
 20133 Milan
 Italy

Gianluca Molla

Università degli Studi
 dell'Insubria
 Dipartimento di Biotecnologie e
 Scienze Molecolari
 Via J.H. Dunant 3
 21100 Varese
 Italy

Bernd Nidetzky

Graz University of Technology
 Institute of Biotechnology and
 Biochemical Engineering
 Petersgasse 12
 8010 Graz
 Austria

Gianluca Ottolina

Istituto di Chimica del
 Riconoscimento Molecolare CNR
 Via Mario Bianco 9
 20131 Milano
 Italy

Lars Haastrup Pedersen

Aalborg University
 Department of Biotechnology
 Chemistry and Environmental
 Engineering
 Sohngårdsholmvej 49
 9000 Aalborg
 Denmark

Francisco J. Plou

Departamento de Biocatálisis
 Instituto de Catálisis y Petroleoquímica
 CSIC
 Cantoblanco
 28049 Madrid
 Spain

Loredano Pollegioni

Università degli Studi dell'Insubria
 Dipartimento di Biotecnologie e
 Scienze Molecolari
 Via J.H. Dunant 3
 21100 Varese
 Italy

Mateja Primožič

University of Maribor
 Faculty of Chemistry and Chemical
 Engineering
 Laboratory for Separation Processes
 and Product Design
 Smetanova 17
 2000 Maribor
 Slovenia

Fred van Rantwijk

Delft University of Technology
 Department of Biotechnology
 Laboratory of Biocatalysis and
 Organic Chemistry
 Julianalaan 136
 2628 BL Delft
 The Netherlands

Jean-Louis Reymond

University of Berne
Department of Chemistry and
Biochemistry
Freiestraße 3
3012 Berne
Switzerland

Sinthuwat Ritthitham

Aalborg University
Department of Biotechnology
Chemistry and Environmental
Engineering
Sohngårdsholmvej 49
9000 Aalborg
Denmark

Felipe Rodríguez-Vico

Departamento de Química-Física
Bioquímica y Química Inorgánica
Edificio CITE I
Carretera de Sacramento S/N
La Cañada de San Urbano
04120 Almería
Spain

Diego Romano

University of Milan
Department of Food Science and
Microbiology
Via Celoria 2
20133 Milan
Italy

Francesco Secundo

Istituto di Chimica del
Riconoscimento Molecolare
CNR
Via Mario Bianco 9
20131 Milano
Italy

Stefano Servi

Politecnico di Milano
Dipartimento CMIC 'G. Natta'
Via Mancinelli 7
20131 Milano
Italy

Roger A. Sheldon

Delft University of Technology
Department of Biotechnology
Laboratory of Biocatalysis and Organic
Chemistry
Julianalaan 136
2628 BL Delft
The Netherlands

Agata Spera

Department of Chemistry
Chemical Engineering and Materials
University of L'Aquila
67040 L'Aquila
Italy

Patrizia Spizzo

Università degli Studi di Trieste
Dipartimento di Scienze Farmaceutiche
Laboratory of Applied and
Computational Biocatalysis
Piazzale Europa 1
34127 Trieste
Italia

Georg A. Sprenger

Universität Stuttgart
Institute of Microbiology
Allmandring 31
70569 Stuttgart
Germany

Haralambos Stamatis

University of Ioannina
Department of Biological Applications
and Technologies
Laboratory of Biotechnology
45110 Ioannina
Greece

Davide Tessaro

Politecnico di Milano
Dipartimento CMIC 'G. Natta'
Via Mancinelli 7
20131 Milano
Italy

Eleni Theodosiou

National Technical University of
Athens
Chemical Engineering
Department
Biotechnology Laboratory
5 Iroon Polytechniou Str.
Zografou Campus
15700 Athens
Greece

Malene S. Thomsen

Research Centre Applied
Biocatalysis
Petersgasse 14
8010 Graz
Austria.

Vojtech Vejvoda

Academy of Sciences of the Czech
Republic
Institute of Microbiology
Center of Biocatalysis and
Biotransformation
142 20 Prague
Czech Republic

Margit Winkler

Technische Universität Graz
Institut für Organische Chemie
Stremayrgasse 16
8010 Graz
Austria

Roland Wohlgemuth

Sigma-Aldrich
Research Specialities
Industriestrasse 25
9470 Buchs
Switzerland

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, Sinthawat 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