Pan Stanford Series
on Biocatalysis
Volume 1

Industrial Biocatalysis

edited by Peter Grunwald

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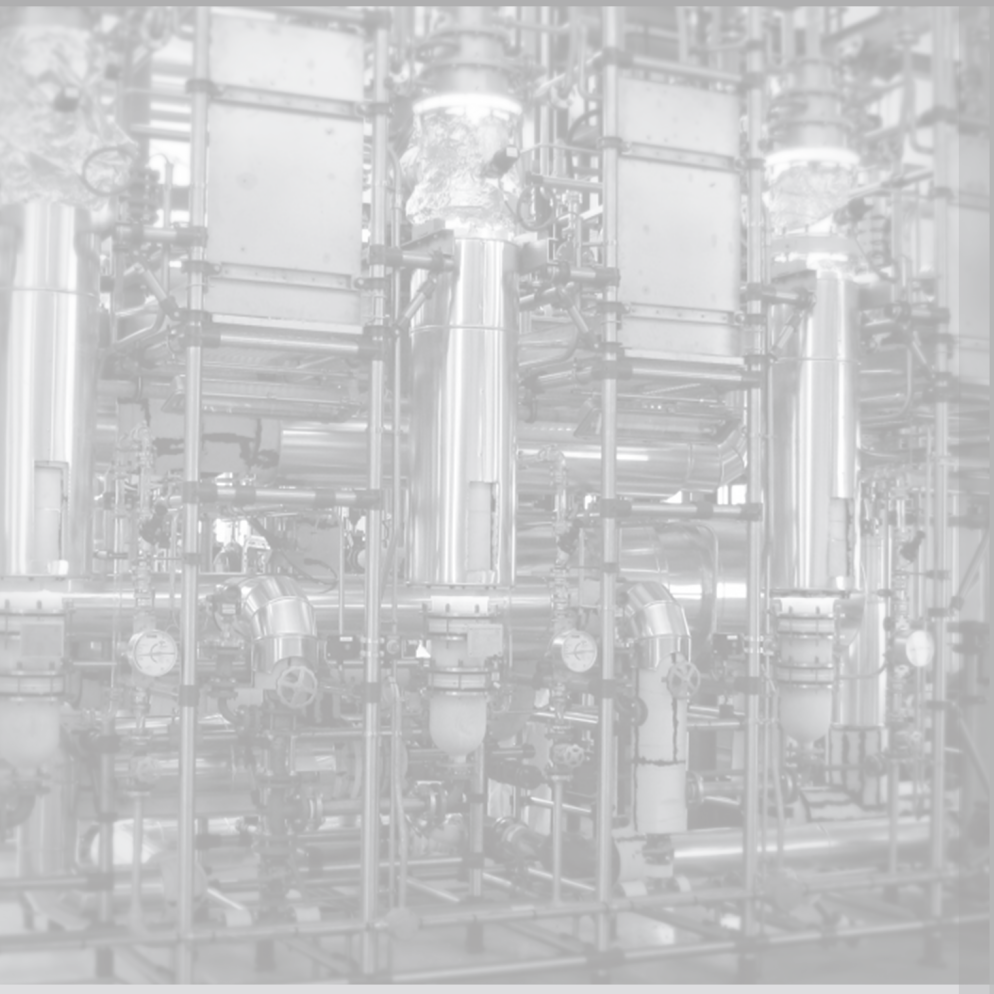

Pan Stanford Series on Biocatalysis

Series Editor
Peter Grunwald

Titles in the Series

Published

Vol. 1
Industrial Biocatalysis
Peter Grunwald, ed.
2015
978-981-4463-88-1 (Hardcover)
978-981-4463-89-8 (eBook)

Forthcoming

Vol. 2
Biocatalysis and Nanotechnology
Peter Grunwald, ed.

Vol. 3
Handbook of Carbohydrate-Modifying Biocatalysts
Peter Grunwald, ed.

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Pan Stanford Publishing

Published by

Pan Stanford Publishing Pte. Ltd.
Penthouse Level, Suntec Tower 3
8 Temasek Boulevard
Singapore 038988

Email: editorial@panstanford.com
Web: www.panstanford.com

British Library Cataloguing-in-Publication Data
A catalogue record for this book is available from the British Library.

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Cover image: Photochemical synthetic step in the production of the antimalarial Artemisinin in Garessio, Italy; by courtesy of Dr. Andreas Burgard, Sanofi.

ISBN 978-981-4463-88-1 (Hardcover)
ISBN 978-981-4463-89-8 (eBook)

Printed in the USA

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Preface

Biocatalysis has meanwhile become an essential tool in the chemical industry and is the central part of biotechnology, defined by the European Federation of Biotechnology already in 1988 as "the integration of natural sciences and organisms, cells, parts thereof, and molecular analogues for products and services." As to the industrial application, biocatalysis is the core of industrial biotechnology, also known as white biotechnology; "white" stands for the positive impact on the environment associated with the use of biocatalysts as enzymes or whole cells in chemical processes as an alternative to chemical catalysts. Drivers of this development are the big challenges resulting from concerns about global climate change and the need for an assured energy supply. These aspects are discussed in Chapter 1 together with an overview of the many areas of daily life where biocatalysts are already employed.

Modern biocatalysis relies to a large extent on the tremendous advances in the so-called "omics techniques" and the structural elucidation of biomolecules, which have led to synthetic biology and metabolic engineering as new research fields with high application potential for the rational design of enzymes and microbial production strains. In Chapter 2, Daniel Meyer and colleagues from the Biotechnology Research and Information Network introduce the reader to strategies for synthetic applications with recombinant whole-cell biocatalysts and metabolically engineered production strains. They see a convergence of both toward an engineering biology that will penetrate all sectors of the chemical industry and forecast the marked launch of a variety of products with properties that outperform their counterparts produced from crude oil with respect to quality and diversity. This is followed by a detailed overview of methods employed for directed evolution of industrial enzymes (creation of libraries and screening) with many examples from all six enzyme classes, written by Youyun Liang, Ee Lui Ang, and Huimin Zhao; Chapter 3 includes directed

evolution in pathway engineering and combinatorial strategies to optimize the expression of pathway genes. The generation of new enzyme functions by mutations is accompanied in many cases by a decrease in thermodynamic stability. In Chapter 4, Nobuhico Tokuriki and colleagues discuss the fundamentals of the underlying processes together with the state of the art concerning methods to overcome such limitations to enable effective enzyme engineering.

Jun-ichiro Hattan and Norihiko Misawa report in Chapter 5 about tailored production strains for the synthesis of functional isoprenoids and the cataloguing of novel terpene synthase genes isolated from edible plants. The contribution by Jordan McEwen and Shota Atsumi (Chapter 6) deals with metabolic engineering applied to the bio-based conversion of CO_2 for the synthesis of new fuel-like molecules as petroleum replacements in the fuel industry. Subsequently, Wael Sabra and An-Ping Zeng address the importance of microbial consortia in industrial biotechnology. In Chapter 7, the authors illustrate how these mixed bacterial cultures can act together to generate bioelectricity (microbial fuel cells), or in the production of hydrogen, methane, and other chemicals.

Eldie Berger, Eloy Ferreras, Mark P. Taylor, and Don A. Cowan critically examine the use of extremophiles in biofuel synthesis; Chapter 8 includes a discussion of major microbial strains and enzymes involved in different production steps, new developments in strain engineering, and pre-treatment procedures for lignocellulosic biomass. In Chapter 9, Aharon Oren describes the potential industrial utilization of halophilic microorganisms and enzymes. Famous examples are the green algae *Dunaliella salina* for producing β-carotene, and *Halomonas elongata* as a source of ectoine that has been reported to act as a "molecular chaperone", and meanwhile found a variety of applications in biotechnology as well as in cosmetic products and in the biomedical area. Till Tiso, Nick Wierckx, and Lars M. Blank review in Chapter 10 the importance and potential of non-pathogenic *Pseudomonas* with a focus on *P. putida* as platform for industrial biocatalysis; the chapter summarizes compounds produced in industry with these microorganisms as well as industrially used enzymes from *Pseudomonas* strains. The chapter contributed by Petra Peters-Wendisch and Volker F. Wendisch (Chapter 11) describes in detail

the use of *Corynebacterium glutamicum* strains for the sustainable production of a variety of compounds such as organic acids, alcohols, diamines, polyhydroxyalkanoates, and proteins from alternative carbon sources, enabled by metabolic engineering.

Chapter 12, contributed by Jesper Brask, David Cowan, and Per Munk Nielsen (Novozymes), deals with the application of industrial enzymes in biodiesel production; the emphasis is on phospholipases used for the removal of phospholipids, and lipases as an alternative to chemical catalysts in the transesterification process.

Enzymes being promiscuous with respect to the substrates accepted or to the catalyzed reaction type are very interesting biocatalysts due to their capability of converting a wide range of substrates. In Chapter 13, Qi Wu and Xian-Fu Lin highlight their (industrial) application in non-conventional organic reactions, including tandem synthetic processes.

Chapters 14 and 15 deal with the immobilization of enzymes, which has a variety of advantages (stabilization, reuse of the catalyst, simplified product recovery, etc.) over the employment of dissolved ones. Julia Stolarow, Berna Gerçe, Christoph Syldatk, Ivana Magario, Christian Morhardt, Matthias Franzreb, and Rudolf Hausmann discuss micro-magnetic non-porous carriers in comparison to porous supports with a focus on theoretical considerations concerning mass transfer phenomena. Oliver Thum, Frank Hellmers (Evonic Industries), and Marion Ansorge-Schumacher provide an overview on the various immobilization strategies together with selected examples of uses of robust immobilized enzymes for large-scale applications under individual process conditions. In Chapter 16, Veronica Stepankova, Jiri Damborsky, and Radka Chaloupkova then treat the behavior of hydrolases in non-conventional media (organic solvents, ionic liquids, deep eutectic solvents, supercritical fluids, and fluorous solvents). By a case study with haloalkane dehalogenases in the presence of organic co-solvents, the authors demonstrate how the effect of solvents on enzyme structure and function can be mechanistically explained at the molecular level and mathematically modeled.

Three contributions deal with members of the enzyme class 1, the oxidoreductases. In Chapter 17, Yilei Fu, Kathrin Castiglione, and Dirk Weuster-Botz report about ene-reductases

from cyanobacteria for industrial biocatalysis. These enzymes are capable of generating chiral molecules by the asymmetric reduction of C=C bonds and are hence of much interest in connection with the synthesis of enantiopure molecules for application in the pharmaceutical and chemical industries. The chapter includes whole-cell bioreductions using engineered *E. coli* overexpressing an ene-reductase. The contribution by Santosh Kumar (Chapter 18) about cytochrome P450 biocatalysts—apart from providing general information about these enzymes—introduces the readers to many applications, including their use for industrial synthesis of drugs, drug metabolites, and other chemicals, for biosensor design to monitor drug levels in the plasma, for gene-directed enzyme prodrug therapy applicable to targeted cancer treatment, and finally for phytoremediation of soil and water contaminants. In Chapter 19, Susana Rodríguez-Couto finally discusses laccases—copper-containing polyphenol oxidases—under the topic "Green Biocatalysts for Greener Applications," which comprises, among others, pulp bleaching, denim finishing, wastewater treatment, delignification of lignocellulosics, and their use for the fabrication of biosensors and biofuel cells. Chapter 20, by Fabian Haitz, Steffen Rupp, Thomas Hirth, and Susanne Zibek, deals with the lipase-catalyzed epoxidation of unsaturated fatty compounds from renewable raw materials, and mineral oil–based linear, branched, or cyclic alkenes as an alternative to chemical procedures. The resulting epoxides are important intermediates in the industrial chemical industry.

The synthetic potential of dihydroxyacetone-utilizing aldolases is the topic of Chapter 21, contributed by Anne K. Samland and Georg A. Sprenger. This rather new group of aldolases catalyzing stereoselective carbon–carbon bond formation is characterized by an unprecedented donor tolerance, a wide acceptor scope, strict stereoselectivity even with unnatural substrates, and temperature and solvent tolerance. Examples of industrial applications are included. Ulrike Engel, Jens Rudat, and Christoph Syldatk (Chapter 22) discuss the "hydantoinase process" with respect to recent developments for the production of non-canonical optically pure amino acids. The isolation of novel strains, optimization by genetic modification, heterogeneous expression and the composition of "designer bugs," and improvement of

space-time yields by process engineering measures like enzyme immobilization have led to a considerable enhancement of the product range.

Dipeptides play a pivotal role in nutrition and fulfill very specific roles within the body. In Chapter 23, Martin Krehenbrink, Ahmed Sallam, and Alexander Steinbüchel discuss possible biotechnological approaches (chemoenzymatic routes, cell-based production methods, etc.) to the production of these compounds with their enormous application potential. The contribution by Torsten Sehl, Justyna Kulig, Robert Westphal, and Dörte Rother deals with synthetic enzyme cascades for the production of valuable 1,2-amino alcohols and 1,2-diols from cheap achiral precursors (Chapter 24) with a focus on stereoselective synthesis of α-hydroxy ketones, vicinal amino alcohols, and diols as versatile building blocks for the pharmaceutical and chemical industries.

Rapamycin is known as a compound with a variety of clinically useful characteristics. Some of these properties have been shown to be potentiated by resveratrol. Both compounds are available from natural sources in only low amounts. In Chapter 25, Victor M. Ye and Sujata K. Bhatia discuss how to increase the availability of these compounds for clinical or nutraceutical applications through the combination of traditional mutagenesis and metabolic engineering.

The subject "Detergent Proteases" (Chapter 26), the use of technical enzymes in the detergent industry, has been covered by Karl-Heinz Maurer (AB Enzymes). The chapter contains all important aspects of this topic, including the market situation, the performance of different proteases, production organisms and processes, product formulations, safety aspects, and environmental risk assessment. Starch is the main carbohydrate component of many agricultural products. Enzymes used in industrial processing of starch, valuable products derived from starch, and its use for the manufacture of biodegradable plastics or for the production of bioethanol are treated in an overview by Józef Synowiecki and coworkers Anna Panek and Olga Pietrow in Chapter 27.

Algae biotechnology belongs to the hot topics in this area: After an introduction to algae as a rich source of energy and high-value products (Chapter 28), Bhavish Patel, Pongsathorn Dechatiwongse and Klaus Hellgardt summarize enzyme-catalyzed

processes in a potential algal biorefinery. The authors describe in Chapter 29 among others key biocatalytic processes leading to the direct excretion of drop-in fuels, ranging from hydrogen and isoprene to long chain hydrocarbons and develop a complete picture of an algae-based biorefinery concept

In Chapter 30, about the biocatalytic synthesis of polymers, Karla A. Barrera-Rivera and Antonio Martinez-Richa introduce the reader to a field of industrial biocatalysis with a promising future because the multitude of different monomers provided by Nature enables in principle the production of polymers with precisely tuned properties. The final chapter provides a brief overview on the development toward an increasing production of bio-based chemicals and materials (commodity chemicals, fine chemicals, polymers), also forecasted by recent market analyses. One of its main drivers is a profound understanding of metabolic pathways, enabling their modification and reconstruction, and the generation of robust "microbial chemical factories."

The contributions compiled in this book underline the highly interdisciplinary character of industrial biocatalysis. Furthermore, they mirror the gradual shift from a primarily petro-based to a more and more bio-based economy—a development where industrial biocatalysis/biotechnology plays a key role in meeting the challenges resulting from the increasing demand of the still continuously growing world population for energy, food and raw materials.

The editor is indebted to the staff at Pan Stanford Publishing Pte. Ltd. for their invaluable support during the publication of this book.

Peter Grunwald