Enzymes in Nonaqueous Solvents

METHODS IN BIOTECHNOLOGY™

John M. Walker, *SERIES EDITOR*

15. **Enzymes in Nonaqueous Solvents: *Methods and Protocols***, edited by *Evgeny N. Vulfson, Peter J. Halling, and Herbert L. Holland, 2001*
14. **Food Microbiology Protocols**, edited by *J. F. T. Spencer and Alicia Leonor Ragout de Spencer, 2000*
13. **Supercritical Fluid Methods and Protocols**, edited by *John R. Williams and Anthony A. Clifford, 2000*
12. **Environmental Monitoring of Bacteria**, edited by *Clive Edwards, 1999*
11. **Aqueous Two-Phase Systems**, edited by *Rajni Hatti-Kaul, 2000*
10. **Carbohydrate Biotechnology Protocols**, edited by *Christopher Bucke, 1999*
9. **Downstream Processing Methods**, edited by *Mohamed A. Desai, 2000*
8. **Animal Cell Biotechnology**, edited by *Nigel Jenkins, 1999*
7. **Affinity Biosensors: *Techniques and Protocols***, edited by *Kim R. Rogers and Ashok Mulchandani, 1998*
6. **Enzyme and Microbial Biosensors: *Techniques and Protocols***, edited by *Ashok Mulchandani and Kim R. Rogers, 1998*
5. **Biopesticides: *Use and Delivery***, edited by *Franklin R. Hall and Julius J. Menn, 1999*
4. **Natural Products Isolation**, edited by *Richard J. P. Cannell, 1998*
3. **Recombinant Proteins from Plants: *Production and Isolation of Clinically Useful Compounds***, edited by *Charles Cunningham and Andrew J. R. Porter, 1998*
2. **Bioremediation Protocols**, edited by *David Sheehan, 1997*
1. **Immobilization of Enzymes and Cells**, edited by *Gordon F. Bickerstaff, 1997*

METHODS IN BIOTECHNOLOGY™

Enzymes in Nonaqueous Solvents

Methods and Protocols

Edited by

Evgeny N. Vulfson

Institute of Food Research, Norwich, UK

Peter J. Halling

University of Strathclyde, Glasgow, UK

and

Herbert L. Holland

Brock University, St. Catharines, Ontario, Canada

Humana Press  **Totowa, New Jersey**

999 Riverview Drive, Suite 208
Totowa, New Jersey 07512

This publication is printed on acid-free paper. ∞
ANSI Z39.48-1984 (American Standards Institute) Permanence of Paper for Printed Library Materials.

Cover design by Patricia F. Cleary.

Production Editor: Mark J. Breaugh.

For additional copies, pricing for bulk purchases, and/or information about other Humana titles, contact Humana at the above address or at any of the following numbers: Tel: 973-256-1699; Fax: 973-256-8341; E-mail: humana@humanapr.com, or visit our Website at www.humanapress.com

Printed in the United States of America. 10 9 8 7 6 5 4 3 2 1

Library of Congress Cataloging in Publication Data

Main entry under title: Enzymes in nonaqueous solvents: methods and protocols.

Methods in molecular biology™.

Enzymes in nonaqueous solvents: methods and protocols./edited by Evgeny N. Wulfson, Peter J. Halling, and Herbert L. Holland.
p. cm.—(Methods in biotechnology; 15)
Includes bibliographical references and index.
ISBN 0-89603-929-3 (alk. paper)
1. Enzymes—Biotechnology. 2. Nonaqueous solvents. I. Vulfson, Evgeny N. II. Halling, Peter J. III. Holland, Herbert L. IV. Series.
TP248.65.E59 E624 2001
6608.29423—dc21 00-025881

PREFACE

Enzymatic catalysis has gained considerable attention in recent years as an efficient tool in the preparation of natural products, pharmaceuticals, fine chemicals, and food ingredients. The high selectivity and mild reaction conditions associated with enzymatic transformations have made this approach an attractive alternative in the synthesis of complex bioactive compounds, which are often difficult to obtain by standard chemical routes. However, the majority of organic compounds are not very soluble in water, which was traditionally perceived as the only suitable reaction medium for the application of biocatalysts. The realization that most enzymes can function perfectly well under nearly anhydrous conditions and, in addition, display a number of useful properties, e.g., highly enhanced stability and different selectivity, has dramatically widened the scope of their application to the organic synthesis.

Another great attraction of using organic solvents rather than water as a reaction solvent is the ability to perform synthetic transformations with relatively inexpensive hydrolytic enzymes. It is worth reminding the reader that in vivo, the synthetic and hydrolytic pathways are catalyzed by different enzymes. However, elimination of water from the reaction mixture enables the "reversal" of hydrolytic enzymes and thus avoids the use of the expensive cofactors or activated substrates that are required for their synthetic counterparts. Also, one should bear in mind that water is by no means an ideal solvent for synthesis; it is relatively expensive to remove on a large scale and it often participates in unwanted side reactions. Thus, the use of enzymes in conventional industrial solvents generally makes it easier and cheaper to incorporate a biotransformation step into the overall synthetic sequence.

Indeed, there are numerous examples of the successful application of enzymes in low water media to industrial-scale production of pharmaceuticals, food ingredients, and fine chemicals.

Methods are very important in any area of research, even more so in a field like nonaqueous biocatalysis, where many methods have been developed relatively recently and have not yet been standardized completely in all laboratories. All too often, the format of standard research papers does not allow methods to be fully described. The importance of key details may be known in the originating laboratory, but may not be appreciated in another, because they cannot be stressed enough, nor reasons explained. The prime objective of *Enzymes in Nonaqueous Solvents* is to address this issue because it was com-

piled to communicate such details. There will also be critical features of methods that are at present not appreciated by anyone, but that may be causing different results in different laboratories. Here again, the fuller presentations in this book should be a basis for the identification of such differences.

For the convenience of the reader, the editors decided to split the submitted material into three parts; broadly, these deal with the biocatalysts, synthetic chemistry, and systems other than just neat organic solvents or solvent mixtures. Those familiar with the subject will no doubt appreciate that such a separation is to a large extent arbitrary and is bound to result in some overlaps. The editors felt, however, that this would provide the book with a certain structure and make it easier for the reader to find specific pieces of relevant information. In addition, each part has a short introduction that surveys the contributions included.

Authors of standard research papers are understandably keen to emphasise their interesting results. Some signs of this can perhaps be detected in contributions to this volume too. As editors, we have tried to encourage authors to include as much detail as possible in describing their methods, and not to dismiss this as rather boring or unnecessary. We hope the result of the authors' efforts will prove valuable to all who are interested in studying or using enzymes in nonaqueous media.

Evgeny N. Vulfson
Peter J. Halling
Herbert L. Holland

CONTENTS

CONTRIBUTORS

PATRICK ADLERCREUTZ • *Department of Biotechnology, Center for Chemistry and Chemical Engineering, Lund University, Lund, Sweden*

MANSOR AHMAD • *Center for Research in Enzyme and Microbial Technology, Fakulti Sains dan Pengajian Alam Sekitar, Universiti Putra Malaysia, Serdang, Malaysia*

MARIA RAQUEL AIRES-BARROS • *Centro de Engenharia Biologica e Quimica, Instituto Superior Tecnico, Lisbon, Portugal*

M. CONCEIÇÃO ALMEIDA • *Instituto de Tecnologia Quimica e Biologica, Universidade Nova de Lisboa, Oeiras, Portugal*

KAMARUZAMAN AMPON • *Universiti Malaysia Sabah, Kota Kinobaler Sabeh, Malaysia*

JOSE A. ARCOS • *Instituto de Catalisis, CSIC, Madrid, Spain*

SUSANA BARREIROS • *Instituto de Tecnologia Quimica e Biologica, Universidade Nova de Lisboa, Oeiras, Portugal*

MAHIRAN BASRI • *Center for Research in Enzyme and Microbial Technology, Fakulti Sains dan Pengajian Alam Sekitar, University Putra Malaysia, Serdang, Malaysia*

GEORGE BELL • *Department of Pure and Applied Chemistry, University of Strathclyde, Glasgow, UK*

PER BERGLUND • *Department of Biotechnology, Royal Institute of Technology, Stockholm, Sweden*

MIKHAIL BORISOVER • *Institute of Soil, Water and Environmental Sciences, The Volcani Center, Bet Dagan, Israel*

EMMANUEL BOURES • *LILICE, Zac "Les Partes de Riom," Riom, France*

MARIE-PIERRE BOUSQUET • *Centre de Bioingenierie Gilbert Durand, Département de Genie Biochimique et Alimentaire, Complexe Scientifique de Rangueil, Toulouse Cedex, France*

JOAQUIM M. S. CABRAL• *Centro de Engenharia Biologica e Quimica, Instituto Superior Tecnico, Lisbon, Portugal*

GIACOMO CARREA • *Istituto di Biocatalisi e Riconoscimento Moleculare, CNR, Milan, Italy*

CRISTINA M. L. CARVALHO • *Centro de Engenharia Biologica Quimica,Instituto Superior Tecnico, Lisbon, Portugal*

SHUI-TEIN CHEN • *Institute of Biological Chemistry, Academia Sinica, Taipei, Taiwan*

Douglas S. Clark • *Department of Chemical Engineering, University of California, Berkeley, CA*
Giorgio Colombo • *Istituto di Biocatalisi e Riconoscimento Molecolare (CNR), Milan, Italy*
Jean-Stéphane Condoret • *Institut National des Sciences Appliquees, Departement de Genie Biochimique et Alimentaire, Toulouse Cedex, France*
David R. Dodds • *Biotransformations Group, Schering-Plough Research Institute, Union, NJ*
Jonathan S. Dordick • *Department of Chemical Engineering, Rensselaer Polytechnic Institute, Troy, NY*
Amélie Ducret • *Microbial and Enzymatic Technology Group, Biotechnology Research Institute, Montréal, Québec, Canada*
Uwe Eichhorn • *Institute of Biochemistry, Leipzig University, Leipzig, Germany*
Johan F. J. Engbersen • *Laboratory of Supramolecular Chemistry and Technology, MESA Research Institute, University of Twente, Enschede, The Netherlands*
Markus Erbeldinger • *Bioscience and Biotechnology Department, University of Strathclyde, Glasgow, UK*
Norhaizan M. Esa • *Center for Research in Enzyme and Microbial Technology, Fakulti Sains dan Pengajian Alam Sekitar, University Putra Malaysia, Serdang, Malaysia*
Patrizia Ferraboschi • *Dipartimento di Chimica e Biochimica Medica, Universitá degli Studi di Milano, Milan, Italy*
Nuno Fontes • *Instituto de Tecnologia Quimica e Biologica, Universidade Nova de Lisboa, Oeiras, Portugal*
Hugo S. Garcia • *Instituto de Catalisis, CSIC, Madrid, Spain*
Lucia Gardossi • *Department of Pharmaceutical Science, Universitá degli Studi, Piazzale Europa, Italy*
Marion Haensler • *Institute of Biochemistry, Leipzig University, Leipzig, Germany*
Peter J. Halling • *Department of Pure and Applied Chemistry, University of Strathclyde, Glasgow, UK*
Jeong Jun Han • *Department of Biological Sciences, Korea Advanced Institute of Sciences and Technology, Taejon, South Korea*
Neil Harper • *Department of Pure and Applied Chemistry, University of Strathclyde, Glasgow, UK*
Erik Hedenström • *Department of Chemistry and Process Technology, Mid Sweden University, Sundsvall, Sweden*

Joseph J. Heijnen • *Kluyver Laboratory for Biotechnology, Delft University of Technology, Delft, The Netherlands*
Charles G. Hill, Jr. • *Instituto de Catalisis, CSIC, Madrid, Spain*
Misao Hiroto • *Department of Biomedical Engineering, Toin Human Science and Technology Center, Toin University of Yokohama, Yokohama, Japan*
Herbert L. Holland • *Department of Chemistry, Brock University, St. Catharines, Ontario, Canada*
Michael J. Homann • *Biotransformations Group, Schering-Plough Research Institute, Union, NJ*
Karl Hult • *Department of Biotechnology, Royal Institute of Technology, Stockholm, Sweden*
Takamitsu Iida • *Department of Material Science and Technology, Faculty of Engineering, Niigata University, Niigata, Japan*
Yuji Inada • *Department of Biomedical Engineering, Toin Human Science and Technology Center, Toin University of Yokohama, Yokohama, Japan*
Hans-Dieter Jakubke • *Institute of Biochemistry, Leipzig University, Leipzig, Germany*
Volker Kasche • *AB Biotechnologie II, Technische Universität Hamburg-Harburg, Hamburg, Germany*
Takuo Kawamoto • *Department of Synthetic Chemistry and Biological Chemistry, Graduate School of Engineering, Kyoto University, Kyoto, Japan*
Romas J. Kazlauskas • *Department of Chemistry, McGill University, Montréal, Québec, Canada*
Jeffrey A. Khan • *Department of Macromolecular Sciences, Institute of Food Research, Reading, UK*
Hideo Kise • *Institute of Materials Science, University of Tsukuba, Ibaraki, Japan*
Natalia L. Klyachko • *Department of Chemistry, Lomonosov Moscow State University, Moscow, Russia*
Yoh Kodera • *Department of Biomedical Engineering, Toin Human Science and Technology Center, Toin University of Yokohama, Yokohama, Japan*
Fragiskos N. Kolisis • *Department of Chemical Engineering, National Technical University, Athens, Greece*
Peter Kuhl • *Institute of Biochemistry, University of Technology Dresden, Dresden, Germany*
Seok Joon Kwon • *Department of Biological Sciences, Korea Advanced Institute of Sciences and Technology, Taejon, South Korea*

Sylvain Lamare • *Laboratoire de Genie Proteique, Université de La Rochelle, La Rochelle Cedex, France*
René Lazaro • *Laboratoire des Aminoacides, Peptides et Proteines LAPP, Université Montpellier 2, Montpellier Cedex, France*
Marie Dominique Legoy • *Laboratoire de Genie Proteique, Université de La Rochelle, La Rochelle Cedex, France*
Andrey V. Levashov • *Department of Chemistry, Lomonosov Moscow State University, Moscow, Russia*
Mike J. J. Litjens • *Kluyver Laboratory for Biotechnology, Delft University of Technology, Delft, The Netherlands*
Robert Lortie • *Microbial and Enzymatic Technology Group, Biotechnology Research Institute, Montréal, Québec, Canada*
David A. MacManus • *Institute of Food Research, Norwich, UK*
Alain Marty • *Centre de Bioingenierie Gilbert Durand, Department de Genie Biochimique et Alimentaire, Complexe Scientifique de Rangueil, Toulouse Cedex, France*
Ayako Matsushima • *Department of Biomedical Engineering, Toin Human Science and Technology Center, Toin University of Yokohama, Yokohama, Japan*
Thierry Maugard • *Centre de Bioingenierie Gilbert Durand, Departément de Genie Biochimique et Alimentaire, Complexe Scientifique de Rangueil, Toulouse Cedex, France*
Lindsey May • *Department of Pure and Applied Chemistry, University of Strathclyde, Glasgow, UK*
Anna Millqvist-Fureby • *Institute of Food Research, Norwich, UK*
Maha M. A. Misbah • *Unilever Research Laboratory, Vlaardingen, The Netherlands*
Pierre Monsan • *Centre de Bioingenierie Gilbert Durand, Departément de Genie Biochimique et Alimentaire, Complexe Scientifique de Rangueil, Toulouse Cedex, France*
Barry D. Moore • *Department of Pure and Applied Chemistry, University of Strathclyde, Glasgow, UK*
Brian Morgan • *Biotransformations Group, Schering-Plough Research Institute, Union, NJ*
Toshiaki Mori • *Department of Biomolecular Engineering, Tokyo Institute of Technology, Yokohama, Japan*
Hiroyuki Nishimura • *Department of Biomedical Engineering, Toin Human Science and Technology Center, Toin University of Yokohama, Yokohama, Japan*

Torbjörn Norin • *Department of Chemistry and Organic Chemistry, Royal Institute of Technology, Stockholm, Sweden*
Shinobu Oda • *Technical Research Laboratory, Kansai Paint Co., Kanagawa, Japan*
Hiromichi Ohta • *Technical Research Laboratory, Kansai Paint Co., Kanagawa, Japan*
Yoshio Okahata • *Department of Biomolecular Engineering, Tokyo Institute of Technology, Yokohama, Japan*
Cristina Otero • *Instituto de Catalisis, CSIC, Madrid, Spain*
Gianluca Ottlina • *Instituto di Biocatalisi e Riconoscimento Moleculare, CNR, Milan, Italy*
Jenny Ottosson • *Department of Biotechnology, Royal Institute of Technology, Stockholm, Sweden*
Johann Partridge • *Department of Pure and Applied Chemistry, University of Strathclyde, Glasgow, UK*
Alan D. Peilow • *Unilever Research Laboratory, Bedford, UK*
Suree Phutrahul • *Department of Chemistry, Faculty of Science, Chiang Mai University, Chiang Mai, Thailand*
Che Nyonya A. Razak • *Center for Research in Enzyme and Microbial Technology, Fakulti Sains dan Alam Sekitar, University Putra Malaysia, Serdang, Malaysia*
Jeffrey A. Reimer • *Department of Chemical Engineering, University of California, Berkeley, CA*
David N. Reinhoudt • *Laboratory of Supramolecular Chemistry and Technology, MESA Research Institute, University of Twente, Enschede, The Netherlands*
Magali Remaud-Simeon • *Centre de Bioingenierie Gilbert Durand, Departément de Genie Biochimique et Alimentaire, Complexe Scientifique de Rangueil, Toulouse Cedex, France*
Joon Shick Rhee • *Department of Biological Sciences, Korea Advanced Institute of Sciences and Technology, Taejon, South Korea*
Joseph O. Rich • *EnzyMed Inc., Iowa City, IA*
Donald A. Robb • *Department of Pure and Applied Chemistry, University of Strathclyde, Glasgow, UK*
Valérie Rolland • *Laboratoire des Aminoacides, Peptides et Proteines LAPP, Université Montpellier 2, Montpellier Cedex, France*
Didier Rotticci • *Department of Chemistry and Organic Chemistry, Royal Institute of Technology, Stockholm, Sweden*

Michael T. Ru • *Department of Chemical Engineering, University of California, Berkeley, CA*
Abu Bakar Salleh • *Center for Research in Enzyme and Microbial Technology Fakulti Sains dan Alam Sekitar, University Putra Malaysia, Serdang, Malaysia*
Enzo Santaniello • *Dipartimento di Chimica e Biochimica Medica, Universitá degli Studi di Milano, Milan, Italy*
Douglas B. Sarney • *Institute of Food Research, Norwich, UK*
Francesco Secundo • *Istituto di Biocatalisi e Riconoscimento Moleculare, CNR, Milan, Italy*
Vasiliki Sereti • *Department of Chemical Engineering, National Technical University, Athens, Greece*
Vladimir Sirotkin • *Department of Chemistry, Kazan State University, Kazan, Russia*
Boris Solomonov • *Department of Chemistry, Kazan State University, Kazan, Russia*
Andrew J. Smallridge • *Department of Chemical Sciences, Victoria University of Technology, Melbourne, Australia*
Boris Solomonov • *Institute of Soil, Water and Environmental Sciences, The Vulcani Center, Bet Dagan, Israel*
Boonyaras Sookkheo • *Institute of Biological Chemistry, Academia Sinica, Tapei, Taiwan*
Antje Spieß • *AB Biotechnologie II, Technische Universität Hamburg-Harburg, Hamburg, Germany*
Haralambos Stamatis • *Department of Chemical Engineering, National Technical University, Athens, Greece*
Adrie J. J. Straathof • *Kluyver Laboratory for Biotechnology, Delft University of Technology, Delft, The Netherlands*
Takeshi Sugai • *Technical Research Laboratory, Kansai Paint Co., Kanagawa, Japan*
Atsuo Tanaka • *Department of Synthetic Chemistry and Biological Chemistry, Graduate School of Engineering, Kyoto University, Kyoto, Japan*
Fritz Theil • *Department of Chemistry, Liverpool University, Liverpool, UK*
Michael Trani • *Microbial and Enzymatic Technology Group, Biotechnology Research Institute, Montréal, Québec, Canada*
Maurie A. Trewhella • *Department of Chemical Sciences, Victoria University of Technology, Melbourne, Australia*
Rein Ulijn • *Department of Pure and Applied Chemistry, University of Strathclyde, Glasgow, UK*
Robert Vail • *Biotransformations Group, Schering-Plough Research Institute, Union, NJ*

Rao H. Valivety • *Department of Pure and Applied Chemistry, University of Strathclyde, Glasgow, UK*
Dirk-Jan van Unen • *Laboratory of Supramolecular Chemistry and Technology, MESA Research Institute, University of Twente, Enschede, The Netherlands*
Evgeny N. Vulfson • *Department of Food Biochemistry and Biotechnology, Institute of Food Research, Norwich, UK*
Kung-Tsung Wang • *Institute of Biological Chemistry, Academia Sinica, Tapei, Taiwan*
Ernst Wehtje • *Department of Biotechnology, Center for Chemistry and Chemical Engineering, Lund University, Lund, Sweden*
Alexandra N. E. Weissfloch • *Chemica Technologies Inc., Bend, OR*
René-Marc Willemot • *Centre de Bioingenierie Gilbert Durand, Department de Genie Biochimique et Alimentaire, Complexe Scientifique de Rangueil, Toulouse Cedex, France*
Aristotelis Xenakis • *Industrial Enzymology Unit, Institute of Biological Research and Biotechnology, The National Hellenic Research Foundation, Athens, Greece*
Tsuneo Yamane • *Laboratory of Molecular Biotechnology, Graduate School of Bio- and Agro-Sciences, Nagoya University, Nagoya, Japan*
Wan Md Zin W. Yunus • *Center for Research in Enzyme and Microbial Technology, Fakulti Sains dan Pengajian Alam Sekitar, University Putra Malaysia, Serdang, Malaysia*
Dimitriy Zakharychev • *Department of Chemistry, Kazan State University, Kazan, Russia*
Aleksey Zaks • *Biotransformations Group, Schering-Plough Research Institute, Union, NJ*