
**BIOREACTOR
IMMOBILIZED
ENZYMES
AND
CELLS
FUNDAMENTALS
AND
APPLICATIONS**

Edited by
MURRAY MOO-YOUNG

ELSEVIER APPLIED SCIENCE

BIOREACTOR IMMOBILIZED ENZYMES AND CELLS

Fundamentals and Applications

Edited by

MURRAY MOO-YOUNG

*Industrial Biotechnology Centre,
University of Waterloo, Ontario, Canada*



ELSEVIER APPLIED SCIENCE
LONDON and NEW YORK

ELSEVIER APPLIED SCIENCE PUBLISHERS LTD
Crown House, Linton Road, Barking, Essex IG11 8JU, England

Sole Distributor in the USA and Canada
ELSEVIER SCIENCE PUBLISHING CO., INC.
52 Vanderbilt Avenue, New York, NY 10017, USA

WITH 77 TABLES AND 179 ILLUSTRATIONS

© ELSEVIER APPLIED SCIENCE PUBLISHERS LTD 1988

British Library Cataloguing in Publication Data

Bioreactor immobilized enzymes and cells:
fundamentals and applications.

1. Immobilized enzymes

I. Moo-Young, Murray

547.7'58 QP601

ISBN 1-85166-160-3

Library of Congress CIP data applied for

No responsibility is assumed by the Publisher for any injury and/or damage to persons or property as a matter of products liability, negligence or otherwise, or from any use or operation of any methods, products, instructions or ideas contained in the material herein.

Special regulations for readers in the USA

This publication has been registered with the Copyright Clearance Center Inc. (CCC), Salem, Massachusetts. Information can be obtained from the CCC about conditions under which photocopies of parts of this publication may be made in the USA. All other copyright questions, including photocopying outside the USA, should be referred to the publisher.

All rights reserved. No part of this publication may be reproduced, stored in a retrieval system, or transmitted in any form or by any means, electronic, mechanical, photocopying, recording, or otherwise, without the prior written permission of the publisher.

88/2/58

生物反应器设计，生物转变，生物转化，生物工艺

生物反应器固定化酶 与细胞

基础与应用

BIOREACTOR IMMOBILIZED ENZYMES AND CELLS

Fundamentals and Applications

PREFACE

Bioreactors are at the heart of biotechnological processes and devices which are used in industrial, agricultural and medical applications. They rely on the bioconversion functions of agents in the form of whole living cells or cell-free enzymes which transform raw materials into useful products and/or less undesirable by-products. In recent years there has been a growing interest in bioreactors which employ 'immobilized enzymes and cells', a phrase now generally used to describe biocatalytic systems in which the bio-agents are segregated and/or attached to solid support carriers in contrast to the more conventional systems in which the bio-agents are in free suspension (cells) or are dissolved in a bulk aqueous medium (enzymes). In principle, there are many relative advantages of the immobilized systems.

This publication is a collection of papers which deals with the generic fundamentals and applications of bioreactor immobilized enzymes and cells, covering the complete range of cell types (animal, plant and microbial cells, including bacteria, yeasts and fungi), various types of bioreactor configurations and operations (fixed and fluidized beds, batch and continuous processes) and various types of immobilized techniques (adsorption, entrapment, encapsulation, compartmentalization). For convenience the material is presented according to the following three sections:

1. Techniques and principles in the preparation and characterization of immobilized cells and enzymes.
2. Applications of these systems in the production of useful products, including pharmaceuticals, foods and chemicals.
3. Applications of these systems in waste treatment and environmental pollution control.

The book is based on manuscripts which have been prepared by some of the world's foremost authorities in this area of biotechnology as well

as by researchers who are becoming known as experts in certain aspects of this area. The material should be useful to students and researchers in industrial biotechnology, biochemical engineering, applied biochemistry and related fields.

In the interest of speed, this volume is produced from camera-ready copies of the original author-generated manuscripts. No attempt has been made to unify the text format in terms of such variables as literature referencing system or symbols nomenclature. In addition, only minimal editorial changes have been made to the usage of the English language in the original manuscript to improve context and clarity. (It should be noted that many of the contributors are from non-English-speaking countries.)

In closing, I wish to record my thanks to Kathy Young, one of my research assistants, who did most of the proof-reading and graphics layout of the manuscripts, and to Penny Preis, my secretary, who handled the final stages of this work. Finally, grateful acknowledgement is made to the Natural Sciences and Engineering Research Council of Canada and UNESCO for financial support of the project.

MURRAY MOO-YOUNG

LIST OF CONTRIBUTORS

S. ADACHI

Applied Microbiology, Department of Agricultural Chemistry, Faculty of Agriculture, Gifu University, Gifu 501-11, Japan

J. AMIOT

Département de Sciences et Technologie des Aliments et Centre de Recherche en Nutrition, Université Laval, Quebec, PQ, Canada G1K 7P4

L. AMOURACHE

Department of Bioengineering, Université de Technologie de Compiègne, France

G. F. ANDREWS

Department of Chemical Engineering, State University of New York at Buffalo, Buffalo, New York 14260, USA

L. A. BEHIE

Department of Chemical Engineering, University of Calgary, Calgary, Alberta, Canada T2N 1N4

H. BERNSTEIN

Department of Chemical Engineering, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, USA

A. BHADRA

Pfizer Ltd, 178 Industrial Area, Chandigarh, India

A. BORCHERT

Fachhochschule Ostfriesland, Emden, Federal Republic of Germany

- E. BRAUER
Universität Frankfurt, Institut für Physikalische und Theoretische Chemie, Federal Republic of Germany
- P. BRODELIUS
Institute of Biotechnology, Swiss Federal Institute of Technology, Hönggerberg, CH-8093 Zürich, Switzerland
- K. BUCHHOLZ
Institut für Landwirtschaftliche Technologie und Zuckerindustrie an der TU Braunschweig, Federal Republic of Germany
- R. CHAMY
Universidad Católica de Valparaíso, Casilla 4059, Valparaíso, Chile
- H. N. CHANG
Korea Advanced Institute of Science and Technology, Seoul, Korea
- T. M. S. CHANG
Artificial Cells and Organs Research Centre, Faculty of Medicine, McGill University, 3655 Drummond Street, Montreal, PQ, Canada
- C. L. COONEY
Department of Chemical Engineering, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, USA
- C. DAUNER-SCHÜTZE
Universität Frankfurt, Institut für Physikalische und Theoretische Chemie, Federal Republic of Germany
- R. C. DEAN JR
Verax Corporation, Lebanon, New Hampshire 03766, USA
- J. DE LA NOUE
Groupe de Recherche en Recyclage Biologique et Aquiculture, Université Laval, Ste Foy, PQ, Canada G1K 7P4
- J. P. FONTA
Department of Chemical Engineering, State University of New York at Buffalo, Buffalo, New York 14260, USA

A. FREEMAN

Department of Biotechnology, Faculty of Life Sciences, Tel-Aviv University, Tel-Aviv 69978, Israel

G. M. GAUCHER

Division of Biochemistry, University of Calgary, Calgary, Alberta, Canada T2N 1N4

K. F. GU

Artificial Cells and Organs Research Centre, Faculty of Medicine, McGill University, 3655 Drummond Street, Montreal, PQ, Canada

T. HAYASHI

Applied Microbiology, Department of Agricultural Chemistry, Faculty of Agriculture, Gifu University, Gifu 501-11, Japan

H. HORITSU

Applied Microbiology, Department of Agricultural Chemistry, Faculty of Agriculture, Gifu University, Gifu 501-11, Japan

A. ILLANES

Universidad Católica de Valparaíso, Casilla 4059, Valparaíso, Chile

S. B. KARKARE

Verax Corporation, Lebanon, New Hampshire 03766, USA

H. W. D. KATINGER

Institute of Applied Microbiology, Vienna, Austria

H. KAUTOLA

Biotechnology and Food Engineering, Department of Chemistry, Helsinki University of Technology, Espoo, Finland

K. KAWAI

Applied Microbiology, Department of Agricultural Chemistry, Faculty of Agriculture, Gifu University, Gifu 501-11, Japan

J. KLEIN

Gesellschaft für Biotechnologische Forschung GmbH, 3300 Braunschweig, Federal Republic of Germany

G. KLEMENT

Institute of Applied Microbiology, Vienna, Austria

R. LANGER

Department of Applied Biological Sciences, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, USA

M. P. MARCHESE

Universidad Católica de Valparaíso, Casilla 4059, Valparaíso, Chile

J. M. MELNYK

St Bonaventure University, Olean, New York, USA

R. A. MESSING

168 Scenic Drive South, Horseheads, New York 14845, USA

H. G. MONBOUQUETTE

Department of Chemical Engineering, North Carolina State University, Raleigh, North Carolina 27695-7905, USA

M. MOO-YOUNG

Department of Chemical Engineering, University of Waterloo, Waterloo, Ontario, Canada N2L 3G1

K. NILSSON

Department of Pure and Applied Biochemistry, University of Lund, POB 124, S-221 99 Lund, Sweden

D. F. OLLIS

Department of Chemical Engineering, North Carolina State University, Raleigh, North Carolina 27695-7905, USA

Y. K. PARK

Universidade Estadual de Campinas, Faculdade de Engenharia de Alimentos (UNICAMP), Campinas 13100, SP, Brazil

G. M. PASTORE

Universidade Estadual de Campinas, Faculdade de Engenharia de Alimentos (UNICAMP), Campinas 13100 SP, Brazil

P. PREMA

Regional Research Laboratory (CSIR), Trivandrum 695019, Kerala, India

D. PROULX

Groupe de Recherche en Recyclage Biologique et Aquiculture, Université Laval, Ste Foy, PQ, Canada G1K 7P4

S. V. RAMAKRISHNA

Regional Research Laboratory (CSIR), Trivandrum 695019, Kerala, India

N. G. RAY

Verax Corporation, Lebanon, New Hampshire 03766, USA

P. W. RUNSTADLER JR

Verax Corporation, Lebanon, New Hampshire 03766, USA

J. M. SCHARER

Department of Chemical Engineering, University of Waterloo, Waterloo, Ontario, Canada N2L 3G1

W. SCHEIRER

Institute of Applied Microbiology, Vienna, Austria

V. P. SREEDHARAN

Regional Research Laboratory (CSIR), Trivandrum 695019, Kerala, India

Y. TAKAHASHI

Applied Microbiology, Department of Agricultural Chemistry, Faculty of Agriculture, Gifu University, Gifu 501-11, Japan

E. J. VANDAMME

Laboratory of General and Industrial Microbiology, University of Ghent, Coupure Links 652, B-9000 Ghent, Belgium

K. VENKATASUBRAMANIAN

Department of Chemical and Biochemical Engineering, Rutgers University, Piscataway, New Jersey 08854, USA

M. A. VIJAYALAKSHMI

Department of Food Science, Université Laval, Quebec, PQ, Canada

J. C. VUILLEMARD

Département de Sciences et Technologie des Aliments et Centre de Recherche en Nutrition, Université Laval, Quebec, PQ, Canada G1K 7P4

R. XIOA

Applied Microbiology, Department of Agricultural Chemistry, Faculty of Agriculture, Gifu University, Gifu 501-11, Japan

V. C. YANG

College of Pharmacy, University of Michigan, Ann Arbor, Michigan 48109, USA

M. E. ZÚÑIGA

Universidad Católica de Valparaíso, Casilla 4059, Valparaíso, Chile

CONTENTS

<i>Preface</i>	v
<i>List of Contributors</i>	xi

Section 1: Techniques and Principles

Matrix design for microbial cell immobilization	1
J. KLEIN	
Structured modeling of immobilized cell kinetics and RNA content	9
H. G. MONBOUQUETTE and D. F. OLLIS	
Analysis of oxygen transport in immobilized whole cells	33
H. N. CHANG and M. MOO-YOUNG	
A large scale membrane reactor system with different compartments for cells, medium and product	53
G. KLEMENT, W. SCHEIRER and H. W. D. KATINGER	
Immobilization of multienzyme system and dextran-NAD ⁺ in semipermeable microcapsules for use in a bioreactor to convert urea into L-glutamic acid	59
K. F. GU and T. M. S. CHANG	
Development of a high capacity adsorbent for enzyme isolation and immobilization	63
C. DAUNER-SCHÜTZE, E. BRAUER, A. BORCHERT and K. BUCHHOLZ	

Nylon filters with rennet enzyme (chymosin) for continuous milk-clotting	71
L. AMOURACHE and M. A. VIJAYALAKSHMI	
The development of an immobilized heparinase reactor	83
V. C. YANG, H. BERNSTEIN, C. L. COONEY and R. LANGER	
Production of biomolecules by immobilized animal cells	95
K. NILSSON	
Urea hydrolysis studies on microbial cells bound to chitin with glutaraldehyde as compared to toluene diisocyanate	101
J. M. MELNYK	

Section 2: Production Applications

Biofilms on adsorbent particles	111
G. F. ANDREWS and J. P. FONTA	
Large-scale culture of hybridoma and mammalian cells in fluidized bed bioreactors	125
R. C. DEAN JR, S. B. KARKARE, N. G. RAY, P. W. RUNSTADLER JR and K. VENKATASUBRAMANIAN	
Effect of water miscible solvents on biotransformations with immobilized cells—an overview	143
A. FREEMAN	
Immobilized plant cells as a source of biochemicals	167
P. BRODELIUS	
The application of continuous three phase fluidized bed bioreactors to the production of pharmaceuticals	197
L. A. BEHIE and G. M. GAUCHER	
Hydrolysis of milk proteins by immobilized cells	213
J. C. VUILLEMARD and J. AMIOT	
Recent progress in the immobilization of β -galactosidase	225
Y. K. PARK and G. M. PASTORE	

Immobilization of lactase and invertase on crosslinked chitin	233
A. ILLANES, M. E. ZÚÑIGA, R. CHAMY and M. P. MARCHESE	
Continuous ethanol production with immobilized yeast cells in a packed bed reactor	251
S. V. RAMAKRISHNA, V. P. SREEDHARAN and P. PREMA	
Immobilized biocatalysts and antibiotic production: biochemical, genetical and biotechnical aspects	261
E. J. VANDAMME	
Production of organic acids by immobilized cells of fungi	287
H. HORITSU, Y. TAKAHASHI, S. ADACHI, R. XIOA, T. HAYASHI, K. KAWAI and H. KAUTOLA	

Section 3: Waste Management Applications

Removal of macronutrients from wastewaters by immobilized microalgae.....	301
D. PROULX and J. DE LA NOUE	
Immobilized cells in anerobic waste treatment	311
R. A. MESSING	
Methane production in immobilized cell bioreactor	317
J. M. SCHARER, A. BHADRA and M. MOO-YOUNG	

MATRIX DESIGN FOR MICROBIAL CELL IMMOBILIZATION

J. Klein

Gesellschaft für Biotechnologische Forschung mbH,

3300 Braunschweig, W. Germany

Introduction

Immobilized cells are now generally accepted as biocatalysts as are immobilized enzymes. This is well documented in the literature (1, 2, 3). In most cases the individual contributions can be arranged in two groups, where the first group refers to papers with main emphasis on immobilization methodology and the second group on a selected microbial biocatalytic reaction or process. On the basis of a number of papers from the authors laboratory belonging to both of the afore mentioned groups it seems justified to draw some more general conclusions on the principles of matrix design for microbial cell immobilization.

There are different routes which could be chosen to structurize this discussion. A first route could give priority to structural parameters, as summarized in Table 1.

Structural Parameters

Chemistry	Precursor Matrix	Toxicity, time scale stability regions hydrophil/phob
Crosslinking density -Porosity		Swelling Permeation, Diffusion Mechanical stability cell growth
Geometry	Size Shape	Transport processes (-O ₂ , substrates, products) -Radius/thickness -bead, fiber, block, film

Table 1: Summary of Structural Parameters Related to Cell Immobilization