Engineering

Edited by E. K. Pye and L. B. Wingard, Jr.

2

ENZYME ENGINEERING
Volume 2

Edited by

E. Kendall Pye
Department of Biochemistry
School of Medicine
University of Pennsylvania
Philadelphia, Pennsylvania

and

Lemuel B. Wingard, Jr.
Department of Pharmacology
School of Medicine
University of Pittsburgh
Pittsburgh, Pennsylvania

PLENUM PRESS • NEW YORK AND LONDON

Library of Congress Cataloging in Publication Data

Engineering Foundation Conference on Enzyme Engineering, 2d, Henniker, N. H., 1973.
Enzyme engineering.

Called volume 2 in continuation of a volume with the same title published in 1972, which contains the papers of the 1st Engineering Foundation Conference on Enzyme Engineering.

1. Enzymes — Industrial applications — Congresses. I. Pye, E. Kendall, ed. II. Wingard, L., ed. III. Title. [DNLM: 1. Biomedical engineering — Congresses. 2. Enzymes — Congresses. W3 EN696]
TP248.E5E53 1973 660'.63 74-13768
ISBN 0-306-35282-6

ACKNOWLEDGMENTS

Permission has been received from the various publishers for reproduction of the following figures.

p. 25, Fig. 2 ***Exp. Cell Res.*** (Academic Press, Inc.)
p. 28, Fig. 5 ***Science*** (Amer. Assoc. Advanc. Sci.)
p. 84-86, Fig. 1, 2, 3 ***Enzyme*** (S. Karger AG, Basel)
p. 84-87, Fig. 1, 2, 4 ***Biotechnol. Bioeng.*** (John Wiley & Sons, Inc.)
p. 118, Fig. 2 ***Nature*** (Macmillan Journals Ltd., London)
p. 226-227, Fig. 1, 2 ***Biotechnol. Bioeng.*** (John Wiley & Sons, Inc.)
p. 344-348, Fig. 1-7 ***Die Starke*** (Verlag Chemie GMBH)
p. 428, Fig. 1 ***J. Polymer Sci.*** (John Wiley & Sons, Inc.)

This volume contains most of the papers and research reports presented at the Second Engineering Foundation conference on Enzyme Engineering held at Henniker, New Hampshire, August 5-10, 1973.

A Division of Plenum Publishing Corporation
227 West 17th Street, New York, N.Y. 10011

United Kingdom edition published by Plenum Press, London
A Division of Plenum Publishing Company, Ltd.
4a Lower John Street, London W1R 3PD, England

Printed in the United States of America

ORGANIZATION OF THE CONFERENCE

Sponsor: The Engineering Foundation
345 East 47th Street
New York, N.Y. 10017 USA

Financial Support:

Corning Glass Works
Corning, N.Y. 14830 USA

and

The Engineering Foundation

Executive Chairman of the Conference: Lemuel B. Wingard, Jr.

Program Chairman of the Conference: E. Kendall Pye

Advisory Board:

Theodore Cayle
Thomas M.S. Chang
Ichiro Chibata
Peter Dunnill
Victor Edwards
Richard Falb
George G. Guilbault
Arthur E. Humphrey
William H. McMullen III
Robert S. Melville
Klaus Mosbach
E. Kendall Pye
Daniel I.C. Wang
Howard H. Weetall
Lemuel B. Wingard, Jr.

ORGANIZATION FOR FUTURE ENZYME ENGINEERING CONFERENCES

Summer 1975 -- Engineering Foundation International Conference tentatively scheduled for USA

Executive Committee:

E. Kendall Pye -- Executive Chairman

Howard H. Weetall -- Program Chairman

Leon Goldstein

Lemuel B. Wingard, Jr.

Advisory Board:

Thomas M.S. Chang	Yehuda Levin
Ichiro Chibata	Georg Manecke
Leland C. Clark, Jr.	David L. Marshall
Clark K. Colton	William H. McMullen
Alkis Constantinides	K. Mosbach
Peter Dunnill	David Novelli
George G. Guilbault	David F. Ollis
William E. Hornby	Hiritoshi Samejima
C.G. Heden	Bern J. Schnyder
Csaba Horvath	P.V. Sundaram
Arthur E. Humphrey	Shuichi Suzuki
D. Jarowek	Daniel I.C. Wang

Summer 1977 -- International Conference tentatively scheduled for Europe

PREFACE

Considerable worldwide interest has arisen in recent years in the controlled use of enzymes as catalysts in industrial processing, analytical chemistry and medical therapy. This interest has generated the new interdisciplinary field of Enzyme Engineering, which includes both the scientific and technologic aspects of the production, purification, immobilization, and application of enzymes in a variety of situations and reactor configurations. A series of Engineering Foundation conferences on Enzyme Engineering was initiated to provide an international forum for the exchange of ideas and information over the entire range of this new field. The outstanding success of the first two conferences attests to the vigor and potential of this field to contribute significantly to a better understanding and resolution of some of the major problems faced by mankind.

The first conference, which was held August 9-13, 1971, at Henniker, New Hampshire, U.S.A., aided significantly in molding the several traditional disciplines that interact to form the field of Enzyme Engineering. The conference was highly successful mainly because many of the key scientists and engineers from the several facets of Enzyme Engineering were brought together for the first time at a single residential meeting. The result was an exchange of ideas and "education" of one another in the pertinent principles of the diverse disciplines which contribute to this field.

The second conference, held August 5-10, 1973, at Henniker, New Hampshire, U.S.A., was equally successful, with 190 participants (from over 350 applicants) attending. Eighteen countries and a wide variety of academic disciplines and occupational specialties were represented. The two major emphases of the 1973 conference were the application of immobilized enzymes and the problems in utilizing enzyme systems that require cofactors.

This volume contains most of the papers and research reports presented at the 1973 conference. In addition the results of a survey taken at the conference and the report of an *ad hoc* committee that met during the conference are included. The names of the session cochairmen are included in the "Table of Contents" in appreciation of their efforts in making the 1973 conference a success.

Many thanks are due to Dr. Sandford S. Cole and his staff at the Engineering Foundation Conferences office in New York for making these conferences possible and to the Corning Glass Works for their continued financial support.

E. Kendall Pye
Lemuel B. Wingard, Jr.

January, 1974

CONTENTS

ENZYME ENGINEERING

DEVELOPMENTS AND CHALLENGE OF ENZYME ENGINEERING

Lemuel B. Wingard, Jr.

Departments of Pharmacology and Chemical Engineering
University of Pittsburgh
Pittsburgh, Pennsylvania

It seems appropriate to start this 1973 conference volume by reflecting on changes in the field since the previous meeting in 1971 and by offering several challenges to stimulate thinking and discussion.

It was very evident in 1971 that the field of enzyme engineering could be characterized as "much potential but little practice". The 1971 conference was the first extended (5 day) international gathering of workers in the several aspects of enzyme engineering and served to introduce the principal biochemists, chemical engineers, microbiologists and others from academia and industry to each other. The biological scientists left the 1971 conference with a better appreciation of the role of diffusion in immobilized enzyme technology; while the engineers left with the message to concentrate more on experimental demonstration and less on abstract mathematical modeling. Each person came away with an appreciation that enzyme engineering was an interdisciplinary field depending on many diverse aspects for ultimate success.

TRENDS SINCE 1971

General interest in enzyme engineering has increased markedly since 1971. At least ten additional conferences or symposia have been held or announced in the interim (Battelle-USA, Rouen-France, NSF Grantee-USA, American Chemical Society-USA, American Institute of Chemical Engineers two meetings-USA, FEBS Dublin-Ireland, Milan-Italy, South Carolina-USA, FEBS Budapest-Hungary). Over 603 papers on immobilized enzymes are scattered through the literature (1), and at least four new books (2-5) plus several reviews (6,7) now are available on enzyme engineering or immobilized enzymes.

Significant government and or industrial funding for enzyme technology research and development is apparent in England, Germany, Italy, Japan, Sweden, USA, and the USSR indicating the very significant international interest in this new area.

The primarily academic enthusiasm of 1971 has been expanded but at the same time made more realistic as numerous industrial firms have begun to take a serious look at the practical applications of enzyme engineering. Some insight as to which industries are seriously looking at enzyme applications can be gained by noting the list of over 350 applicants to attend this 1973 conference. Very significant interest is shown by the sugar and corn syrup manufacturers, by clinical diagnostic and medical device manufacturers, by pharmaceutical companies, by enzyme manufacturing and separation companies, and by groups involved in blood chemistry and blood fractionation. At least in the USA many of the largest chemical companies appear to be only mildly if at all concerned with potential applications of immobilized enzymes; although some have small research groups to keep familiar with advances in the field.

In the academic sector many more universities and many more disciplines have joined the ranks of those investigating the basic chemistry and microbiology, engineering considerations, and potential applications of enzyme engineering. Several of the more novel developments or approaches include fluidized bed reactor studies (8), immobilized immunoproteins (9), immobilization on animal tissue cell walls and membranes (10), immobilized mixed function oxidases (11), immobilized clotting and fibrinolysis factors (12), degradation of viruses by immobilized enzymes (13), carbohydrate synthesis with immobilized enzymes (4), and enzymes in nonaqueous environments (13).

At present immobilized enzymes are used commercially in Japan to resolve a racemic mixture of D and L amino acids and in Europe and the USA for inhouse pharmaceutical manufacture of penicillins and steroids. Glucose isomerase, immobilized in microbial cells, is in commercial use in the USA, and a process using the same enzyme immobilized on a more conventional inert support is very close to commercialization. Glucose isomerase catalyzes the conversion of glucose to fructose; the resulting product, originating from cornstarch, appears to be a strong competitor to sucrose-derived invert sugar as the primary commercial sweetening agent in foods and beverages. Similarly, the commercial use of immobilized lactase to remove lactose from whey should appear soon. A number of analytical devices incorporating immobilized enzymes on pads and in hollow tubes and electrodes are in the process of commercial evaluation and introduction, especially in clinical chemistry. And, finally, medical uses of immobilized enzymes have remained primarily as research tools with only a very few clinical trials involving encapsulated enzymes and extracorporeal perfusion through immobilized enzyme tubes.